Vaccine-preventable diseases in childhood, adolescence, and young adulthood in Iceland – rotavirus, meningococci, and influenza: Room for improvements?

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Thesis for the degree of Philosophiae Doctor

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Valtýr Stefánsson Thors

Doctoral committee

Karl G. Kristinsson
Magnús Gottfreðsson
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Sjúkdómar sem fyrirbyggja má með bólusetningum hjá börnum, unglingum og ungu fólki á Íslandi – rótaveira, meningókokkar og inflúensa: Er þörf á umbótum?

Íris Kristinsdóttir

Ritgerð til doktorsgráðu

Leiðbeinendur

Ásgeir Haraldsson
Valtýr Stefánsson Thors

Doktorsnefnd

Karl G. Kristinsson
Magnús Gottfreðsson
Þórólfur Guðnason

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LÆKNADEILD

HÁSKÓLI ÍSLANDS

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ORCID: 0000-0002-8869-9332

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Ágrip

Bólusetningar eru meðal merkustu uppgötvana í sögu læknavísindanna. Með þeim hefur fjölmörgum lífum verið bjargað og hafa áður skæðir smitsjúkdómar nánast horfið eftir tilkomu bólusetninga. Mikilvægt er að heilbrigðisyfirvöld séu vel vakandi fyrir tilkomu nýrra bóluefna, sem og breytingum á faraldsfræði smitsjúkdóma sem kallað gætu á breytingar á bólusetningarskema. Þar að auki er afar mikilvægt að almenn þátttaka í bólusetningum sé góð og að þeir hópar sem eru í mestri hættu á að veikjast alvarlega séu verndaðir eins og hægt er.

Markmið doktorsverkefnisins var að meta hvort þörf sé á umbótum á bólusetningum á Íslandi, með tilliti til bólusetninga gegn rótaveiru, meningókokkum og inflúensu. Doktorsverkefnið samanstendur af fjórum rannsóknum.

Rannsókn I fjallaði um sjúkdómsbyrði rótaveiru hjá ungum börnum á Íslandi og kostnaðarhagkvæmni þess að bæta rótaveirubólusetningum við íslenskt bólusetningarskema. Framskyggn, tveggja ára rannsókn var gerð á Bráðamóttöku Barnaspítala Hringsins. Börnum undir sex ára aldri sem leituðu þangað vegna bráðra garnasýkinga var boðin þátttaka í rannsókninni. Saursýnum var safnað frá þátttakendum og upplýsingum safnað um heildarlengd veikinda, þörf á meðferð á bráðamóttöku barna og innlögn á sjúkrahús, dagafjölda sem foreldrar misstu frá vinnu og fleira. Foreldrar voru einnig spurðir um afstöðu sína til rótaveirubólusetninga. Niðurstöður rannsóknarinnar leiddu í ljós að rótaveira er algengasta veiran í bráðum garnasýkingum hjá ungum börnum sem leiðir til komu á bráðamóttöku barna. Rótaveira veldur umtalsverðri sjúkdómsbyrði á ung börn, foreldra þeirra og heilbrigðiskerfið, sem leiðir til töluverðs vinnutaps foreldra. Vinnutapið er stærsti samfélagslegi kostnaðurinn sem hlýst af bráðum garnasýkingum af völdum rótaveiru. Meirihluti foreldra er hlynntur notkun rótaveirubóluefna, auk þess sem rótaveirubólusetningar á Íslandi væru kostnaðarhagkvæmar. Við ályktum sem svo að bæta ætti rótaveirubólusetningum í bólusetningarskema barna á Íslandi.

Markmið rannsóknar II var að meta algengi einkennalausrar meningókokka-sýklunar í þremur aldurshópum (hjá leikskólabörnum, 10. bekkingum og menntaskólanemum á aldrinum 18-20 ára), kortleggja algengi mismunandi hjúpgerða meningókokka í einkennalausri sýklun og hve lengi

sýklun meningókokka varir. Nefkoksstrokum var safnað frá leikskólabörnum og hálsstrokum frá 10. bekkingum og menntaskólanemum á vormánuðum 2019. Engir meningókokkar greindust í 460 sýnum frá leikskólabörnum. Af sýnum frá 10. bekkingum var eitt jákvætt fyrir meningókokkum (1/197, 0,5%) og 34 frá menntaskólanemum (34/525, 6,5%). Flestir meningókokkar sem greindust voru óhjúpaðir (hjá 26/35 berum). Af hjúpuðum meningókokkum voru meningókokkar B algengastir (hjá 6/35 berum). Tveir einstaklingar báru meningókokka Y og einn meningókokka W. Enginn greindist með meningókokka C, þá týpu sem bólusett er gegn á Íslandi. Lengsta tímalengd sýklunar í rannsókninni var 21 mánuður (frá fyrsta jákvæða sýni til síðasta jákvæða sýnis). Meningókokkar sem greindust við endurteknar sýnatökur í langvarandi sýklun voru náskyldir, sem bendir til viðvarandi sýklunar með sama stofni. Niðurstöður rannsóknarinnar sýna að beratíðni meningókokka, sérstaklega hjúpaðra meningókokka, er lág á Íslandi. Í ljósi þess að nýgengi ífarandi meningókokkasýkinga er einnig lágt er ekki þörf á breytingum á bólusetningum gegn meningókokkum á Íslandi að svo stöddu.

Í rannsókn III var vessabundið og frumubundið ónæmissvar við inflúensubólusetningu borið saman á milli unglinga með offitu og unglinga í kjörþyngd. Þrjátíu unglingar með offitu og þrjátíu unglingar í kjörþyngd tóku þátt í rannsókninni. Þátttakendur voru bólusettir með fjórgildu inflúensu-bóluefni. Blóðprufur voru teknar fyrir bólusetningu og aftur fjórum vikum eftir bólusetningu. Niðurstöður rannsóknarinnar benda til þess að bæði vessabundin og frumubundin svörun við inflúensubólusetningu sé sambærileg á milli hópanna tveggja og því ekki þörf á breytingum á inflúensubólusetningum unglinga með offitu.

Rannsókn IV fjallaði um inflúensubólusetningar barnshafandi kvenna yfir tíu inflúensu-tímabil og sjúkdómsbyrði inflúensu hjá barnshafandi konum og ungbörnum þeirra. Rannsóknin náði til inflúensu-tímabilanna 2010-2020. Bólusetningarhlutfallið var lægst 6,2% á inflúensu-tímabilinu 2011-2012 en hækkaði yfir tímabilið og var hæst 37,5% á tímabilinu 2019-2020. Inflúensubólusetning á meðgöngu er verndandi gegn inflúensu á viðkomandi inflúensu-tímabili en virkni bóluefnisins var 34-100% eftir tímabilum. Inflúensubólusetning á meðgöngu ver einnig ungbörn gegn inflúensu á því tímabili sem móðir er bólusett og veitir líklega vernd gegn inflúensu á fyrstu sex mánuðum lífs.

Lykilorð:

Bólusetningar; Rótaveira; *Neisseria meningitidis*; Inflúensa

Abstract

Vaccines are among the most important medical discoveries ever made and are the cornerstone of public health interventions. Public health authorities must be attentive to the local epidemiology of infectious diseases and the benefits of changing the immunisation schedule and introducing new vaccines into the national immunisation programmes. The aims of the thesis were to assess whether changes and improvements are needed to the Icelandic national immunisation programme regarding immunisations against rotavirus, *Neisseria meningitidis* and influenza. The thesis is comprised of four studies.

In study I, the disease burden of rotavirus in young children in Iceland was assessed, as well as the cost-effectiveness of including rotavirus vaccinations in the national immunisation programme in Iceland. A two-year prospective study was conducted. Children under the age of six attending a paediatric emergency department with acute gastroenteritis were recruited. Stool samples were collected from participants, as well as information about the total duration of symptoms, the need for treatment in the emergency department and for hospital admissions, the number of days parents missed from work etc. Parents were also asked about their opinion on rotavirus vaccinations. Study I showed that rotavirus is the most common virus causing acute gastroenteritis leading to emergency department visits in young children in Iceland. Rotavirus causes a significant disease burden on young children, their parents, and the health care system. A substantial loss of productivity is attributable to rotavirus acute gastroenteritis, leading to considerable societal costs. The addition of a rotavirus vaccine to the national immunisation programme would be cost-effective and most parents are in favour of it. We conclude that rotavirus vaccines should be added to the national immunisation programme.

Study II assessed the prevalence of asymptomatic meningococcal carriage in children, adolescents and young adults in Iceland, the prevalence of meningococcal capsular groups in colonisation and the duration of colonisation. Nasopharyngeal swabs were collected from 1–6-year-old children in daycare centres and oropharyngeal swabs from 15–16-year-old adolescents and 18–20-year-old young adults in the spring of 2019. Of 460 children, none carried meningococci. The colonisation prevalence was 0.5%

among adolescents (1/197) and 6.5% among young adults (34/525). Non-groupable meningococci were most common in colonisation (26/35 carriers), followed by MenB (6/35 carriers). Two participants carried MenY and one carried MenW. No carriage of MenC, the group that is currently vaccinated against in Iceland, was detected. The longest duration of carriage was 21 months (from the first positive swab to the last positive swab). Meningococcal isolates from successive swabs from the same carrier were closely related, indicating a prolonged carriage with the same strain. Study II shows that meningococcal colonisation prevalence, especially of capsulated meningococci, is low in Iceland. Considering both the low colonisation prevalence and low incidence of invasive meningococcal disease, we conclude that changes in meningococcal vaccination strategies in the national immunisation programme are not currently needed.

In study III, the humoral and cellular immune responses to influenza vaccinations were assessed in adolescents with obesity compared to adolescents with normal weight. Thirty adolescents with obesity and thirty adolescents with normal weight were recruited. The participants were vaccinated with a tetravalent influenza vaccine. Venous blood samples were collected before vaccination and again four weeks after vaccination. The results of study III show that both humoral and cellular immune responses to influenza vaccination are similar in adolescents with obesity and adolescents with normal weight. Therefore, changes are not needed to influenza vaccination methods for adolescents with obesity.

Study IV assessed the influenza vaccine uptake in pregnant women in the ten influenza seasons 2010-2020, and the burden of influenza on pregnant women and their infants. The influenza vaccine uptake ranged from 6.2% in 2011-2012 to 37.5% in 2019-2020. Vaccinations against influenza in pregnancy protect pregnant women and their infants in the season of vaccination and provide probable protection for infants <6 months of age. The uptake of influenza vaccinations among pregnant women was suboptimal. Initiatives are needed to increase awareness about the safety and the benefits of influenza vaccinations, to increase influenza vaccine uptake and protect these vulnerable groups against influenza.

Keywords:

Vaccinations; Rotavirus; *Neisseria meningitidis*; Influenza

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Contents

List of abbreviations

4CMenB	Four component meningococcal B vaccine
AGE	Acute gastroenteritis
aOR	Adjusted odds ratio
BAST	Bexsero Antigen Sequence Typing
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BSA	Bovine serum albumin
CC	Clonal complex
CD	Cluster of differentiation
CDC	Centers for Disease Control and Prevention
CFR	Case fatality rate
cgMLST	Core genome multilocus sequence typing
CI	Confidence interval
cnl	capsule null locus
Cq	Quantification cycle
DCC	Daycare centre
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
ED	Emergency department
EEA	European Economic Area
EU	European Union
FACS	Fluorescence-Activated Cell Sorting
FBS	Foetal bovine serum
fHbp	Factor H binding protein
GMT	Geometric mean titre
GP	General practice
HA	Haemagglutinin

HAI	Haemagglutination inhibition
HAU	Haemagglutination Units
ICD-10	International Classification of Disease, version 10
ICU	Intensive care unit
IFN-γ	Interferon γ
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
ILI	Influenza-like illness
IMD	Invasive meningococcal disease
IR	Incidence rate
IRR	Incidence rate ratio
ISK	Icelandic kronur
i.v.	Intravenous
MALDI-TOF MS	Matrix-assisted laser desorption ionisation-time of flight mass spectrometry
MenA	Meningococcal group A
MenB	Meningococcal group B
MenC	Meningococcal group C
MenDeVAR	Meningococcal Deduced Vaccine Antigen Reactivity
MenW	Meningococcal group W
MenY	Meningococcal group Y
MLST	Multilocus sequence typing
MN	Microneutralization
NA	Neuraminidase
NadA	Neisseria adhesin A
NBCS	Newborn calf serum
NG	Non-groupable
NHBA	Neisserial heparin binding antigen
NHS	The National Health Service (UK)

NICU	Neonatal intensive care unit
NIP	National immunisation programme
Nm	*Neisseria meningitidis*
NSP	Non-structural protein
OPS	Oropharyngeal swab
OMV	Outer membrane vesicle
OR	Odds ratio
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PHE	Public Health England
p.o.	Per os
qPCR	Quantitative polymerase chain reaction
RBCs	Red blood cells
RDE	Receptor Destroying Enzyme
RNA	Ribonucleic acid
RT	Room temperature
RSV	Respiratory syncytial virus
RV	Rotavirus
RVAGE	Rotavirus acute gastroenteritis
SD	Standard deviation
ST	Sequence type
TNF-α	Tumour necrosis factor α
UK	United Kingdom
USA	United States of America
VE	Vaccine effectiveness
VP	Viral protein
WGS	Whole genome sequencing
WHO	World Health Organization

List of figures

List of tables

List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-IV):

I. **Kristinsdottir I**, Haraldsson A, Löve A, Asgeirsdottir TL, Thors V. Burden of rotavirus disease in young children in Iceland – Time to vaccinate? *Vaccine.* 2021;39(38):5422-5427. DOI: 10.1016/j.vaccine.2021.07.053

II. **Kristinsdottir I**, Visser LJ, Miellet WR, Mariman R, Pluister G, Haraldsson G, Haraldsson A, Trzciński K, Thors V. Meningococcal carriage in Icelandic children and young adults: a cross-sectional and longitudinal study, Iceland, 2019 to 2021. *Eurosurveillance.* 2023;28(39):pii=2300215. DOI:10.2807/1560-7917.ES.2023.28.39.2300215

III. **Kristinsdottir I**, Haraldsson A, Brynjolfsson SF, Helgason T, Ludviksson BR, Gianchecchi E, Razzano I, Montomoli E, Thors V. Obesity in adolescents does not influence early immune responses to influenza vaccination. *Infectious Diseases.* 2023; 55:6, 415-424. DOI: 10.1080/23744235.2023.2195491

IV. **Kristinsdottir I,** Haraldsson A, Thors V. Influenza vaccine uptake in pregnant women in Iceland 2010-2020 and the burden of influenza in pregnant women and their infants. *Submitted for publication.*

In addition, some unpublished data is presented.

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Declaration of contribution

Study I – The RICE study: The doctoral student, Íris Kristinsdóttir (ÍK), analysed and interpreted the data; gathered information for the cost-effectiveness analysis and did the analysis with the co-authors. ÍK drafted the initial manuscript and critically reviewed and edited the manuscript with the co-authors.

Study II – The MENICE study: ÍK designed the study, together with the co-authors and wrote the study protocol with Ásgeir Haraldsson (ÁH) and Valtýr Thors (VT). She collected all samples from the children attending day-care centres and collected samples from adolescents and young adults during school visits with VT, ÁH and nurses. ÍK coordinated all follow-up visits and collected all samples. ÍK contributed substantially to the plating of the samples, re-culturing of frozen strains, and contributed to the DNA extraction. ÍK carried out the biochemical testing on meningococcal isolates. She analysed and interpreted the data with the collaborators. ÍK drafted the initial manuscript and revised and edited the manuscript with the co-authors.

Study III – The OFICE study: ÍK designed the study with the co-authors and wrote the protocol with ÁH and VT. She coordinated the study, planned and carried out all study visits, collected the clinical data, analysed and interpreted the data, drafted the initial manuscript and revised and reviewed the manuscript.

Study IV – The FluRisk study: ÍK designed the study and wrote the study protocol with ÁH and VT, did the data analysis and interpreted the data. ÍK drafted the initial manuscript and revised and reviewed the manuscript.

This **thesis** was written by the doctoral student.

1 Introduction

1.1 Vaccines

Vaccinations are among the most important medical discoveries ever made and remain the cornerstone of modern public health interventions. The earliest reports of immunisations against infectious diseases are from China in A.D. 1000. Smallpox lesions were powdered and snorted, or fluid was extracted from smallpox pustules and rubbed onto the scratched skin of those that should be protected.[1] The method, sometimes referred to as variolation, spread to the Ottoman Empire and from there to Europe. Lady Mary Wortley Montagu introduced variolation to Europe. She came to know the method in Constantinople and had her son variolated. After returning to London, she advocated for variolations, and the first variolation in England was done on Lady Mary's daughter in 1721.[1,2]

In 1796, a breakthrough in protection against infectious diseases occurred when Edward Jenner inoculated a boy with matter from cowpox lesions to protect against smallpox.[1] This was the beginning of the development of vaccines. Around a century later, Louis Pasteur created the first laboratory-made vaccine, a live attenuated vaccine against *Vibrio cholera* and shortly thereafter against anthrax.[1] Since then, numerous vaccines have been developed against various infectious diseases. The World Health Organization (WHO) estimates that immunisations prevent 3.5-5 million deaths annually.[3] However, worldwide vaccine coverage is an ongoing challenge. Therefore, GAVI, the Vaccine Alliance, was established in 2000 to increase access to immunisations for children in low-income countries.[4,5] In 20 years, from 2000 to the end of 2020, more than 888 million children were vaccinated through GAVI-supported vaccination programmes.[6]

The COVID-19 pandemic negatively affected childhood vaccinations; the number of entirely unvaccinated children increased by 5 million from 2019 to 2021.[7] The COVID-19 pandemic did, however, prove once more how effective vaccinations are in combating infectious diseases, with rapid developments of new vaccines and millions of lives saved with vaccinations.[8,9]

Despite the enormous benefit of vaccinations, there is room for improvement, and vaccines against many existing and emerging infectious

diseases have yet to be developed. Furthermore, public health authorities must be vigilant regarding the local epidemiology of infectious diseases and keep the national immunisation programmes (NIP) up to date with the introduction of new vaccines or changes in vaccination strategies against certain infectious diseases. In this thesis, some potential needs for improvements in the Icelandic NIP are studied, namely whether rotavirus vaccines should be added to the immunisation schedule, whether meningococcal vaccination strategies should be changed, if influenza vaccinations adequately protect adolescents with obesity and whether pregnant women are vaccinated against influenza according to guidelines.

1.1.1 The major types of vaccines

Vaccines are usually classified by their composition. The major types of vaccines are live attenuated vaccines, inactivated vaccines (killed whole organisms), toxoid vaccines, subunit vaccines and protein-polysaccharide conjugate vaccines.[1,10] More recently, nucleic acid vaccines and viral vector vaccines were developed and introduced.[10]

Live attenuated vaccines are usually used against viruses, for example, rotavirus, varicella, measles, and mumps.[1,10] The virus is attenuated by passing it through repeated cultures, resulting in an accumulation of mutations that reduce the virus's ability to cause an infection.[11] With live attenuated vaccines, the pathogen replicates enough within the host to elicit an immune response but not enough to cause disease.[10,11] However, in immunocompromised hosts, the attenuated pathogen can replicate so much that it may cause disease, and these types of vaccines are, therefore, not indicated in this group.[10]

Subunit vaccines include a part of the microbe, often a polysaccharide or a protein. Adjuvants are frequently used with non-live vaccines to elicit a stronger immune response.[10] Inactivated influenza vaccines and vaccines against hepatitis viruses A and B are examples of subunit vaccines.[1,10] Polysaccharide subunit vaccines do not elicit a T cell response since polysaccharides are T cell-independent antigens.[10] By conjugating the polysaccharide to a protein carrier (often a tetanus toxoid protein), the vaccine produces a potent antibody response by using both T cell-dependent (protein) and T cell-independent (polysaccharide) antigens.[10] That leads to long-lasting antibody production and a better immune response in infants.[10] Vaccines against pneumococci and meningococcal capsular groups A, C, W and Y are examples of protein-polysaccharide conjugate vaccines. Toxoid vaccines are used against diphtheria and tetanus.[10] Viral vector vaccines,

using adenovirus as a vector for selected antigens, have been licenced against SARS-CoV-2 and Ebola virus.[12] The first mRNA vaccines were licensed against SARS-CoV-2 during the COVID-19 pandemic. They were found to be generally safe and effective.[13,14] However, an increased risk of myocarditis in adolescent and young adult males was observed after vaccinations.[15] mRNA vaccines against influenza are being developed, but none have been licenced yet.[16]

Vaccines are generally very safe, although, like with all medical interventions, there are some risks of adverse effects.[17,18] Most adverse effects are mild and short-lived, such as local reactions at the injection site, headache, fever, and malaise.[13,14,19-21] Other adverse effects are rare.[18] They include allergic reactions to the vaccine, idiopathic thrombocytopenic purpura, febrile seizures and Guillain-Barré syndrome.[17,18] However, the benefits of vaccinations outweigh the small risk.

This thesis will focus on rotavirus, *Neisseria meningitidis* and influenza, the vaccine-preventable diseases caused by these pathogens and the potential for improved vaccination strategies against them in the Icelandic national immunisation programme.

1.2 Rotavirus

1.2.1 The rotavirus

Rotavirus (Figure 1) was first described in the 1970s when a virus was identified in duodenal biopsies and stool samples from children suffering from non-bacterial acute gastroenteritis.[22-24] The virus belongs to the family *Reoviridae* and is an icosahedral, non-enveloped double-stranded RNA (dsRNA) virus.[25] The genome consists of 11 double-stranded RNA segments that encode for structural and non-structural viral proteins.[25] There are six structural viral proteins (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5,

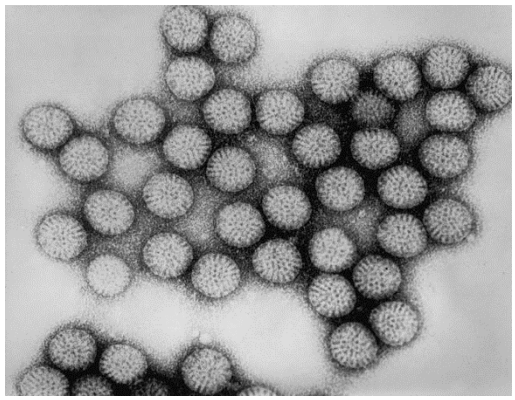

Figure 1. *Rotavirus.*
Rotavirus particles, as seen in a transmission electron microscope. Source: CDC. https://phil.cdc.gov/Details.aspx?pid=273

NSP6).[25,26] The structural capsid proteins (VPs) determine the antigenic properties and the serotype of the virus. The non-structural proteins have a role in the pathogenesis and viral replication.[26] Ten different rotavirus species have been described (A-J) based on the VP6 protein.[25] Rotavirus species A is the most common species in human infection and the one that is usually referred to as rotavirus. It is classified into genotypes based on a glycoprotein (G, VP7) and a protease-cleaved protein (P, VP4) in the outer capsid. The genotypes are referred to by the numbers of these two proteins, e.g., G1P[8].[25]

1.2.2 Rotavirus acute gastroenteritis

Rotavirus infections present with non-bloody diarrhoea, vomiting, fever and malaise.[25] Transmission occurs via the faecal-oral route,[25] and the incubation time is one to three days.[27] Rotavirus is shed in copious amounts in the stool of infected individuals, but only a few virus particles are needed to infect a non-immune person.[28] Recurrent infections can occur, but disease severity usually diminishes with recurrent infections,[29] and children are seldomly hospitalised more than once due to rotavirus acute gastroenteritis (RVAGE).[30]

RVAGE is a self-limiting disease. The management is supportive, preventing or treating dehydration and electrolyte imbalances that might arise with the illness.[25] Oral rehydration solutions are recommended as first-line fluid and electrolyte management, with intravenous fluids given in more severe cases. Anti-emetics, such as ondansetron or metoclopramide, can be used in cases with severe vomiting, and they reduce the need for intravenous fluids, although they can increase diarrhoea.[25]

The Vesikari score is often used to determine RVAGE severity.[31] One to three points are given for signs and symptoms of AGE, as shown in Table 1, resulting in scores that range from 0 to 20.[31] Higher scores denote a more severe disease.[31]

Table 1. *The Vesikari score.*
Points from 0 to 3 are assigned for each sign and symptom listed in the table, and the points are added together to give the Vesikari score. Higher scores denote a more severe disease.

Parameter | Points	**0**	**1**	**2**	**3**
Diarrhoea duration, days	0	1-4	5	≥6
Max. number of diarrhoeal stools/24 hours	0	1-3	4-5	≥6
Vomiting duration, days	0	1	2	≥3
Max. number of vomiting episodes/24 hours	0	1	2-4	≥5
Max. recorded fever, °C	<37	37.1-38.4	38.5-38.9	≥39
Dehydration			1-5%	≥6%
Treatment	None	Rehydration	Hospitalisation	

1.2.3 Epidemiology and burden of rotavirus disease

Rotavirus is one of the major pathogens causing acute gastroenteritis (AGE) in young children,[32-35] and most children have had a rotavirus infection by five years of age.[29,36,37] Rotavirus acute gastroenteritis has been reported as the cause of 1-4% of all ED visits among children under the age of five and a third of all ED visits due to community-acquired AGE.[38] Deaths attributable to rotavirus among children <5 years old were estimated to be around 50 in 2016 (95% uncertainty interval 38-64) in Western Europe, with a mortality rate of 0.2/100,000 children <5 years old.[39] Even though mortality due to rotavirus illness is rare in high-income countries, rotavirus is still the most common cause of death due to diarrheal illness in high-income countries, accounting for 51% of deaths due to diarrhoea in Denmark and 62.6% in Finland.[39] Furthermore, it places a substantial burden on children, families, health care systems and society in the form of the illness itself, reduced quality of life during the RVAGE episode, general practice (GP) and ED visits, hospital admissions and loss of productivity during the time parents take care of their ill children.[39-43] RVAGE follows a seasonal pattern and is most prominent in late winter and early spring,[32,37,44,45] often coinciding with seasonal influenza and respiratory syncytial virus (RSV) epidemics,[45-47] further increasing the strain on healthcare facilities. The main complications

of RVAGE are dehydration, electrolyte disturbances and seizures.[30,35,48] Many RVAGE-associated seizures are afebrile, occur without electrolyte disturbances and are generally benign.[30,48]

1.2.4 Rotavirus vaccinations

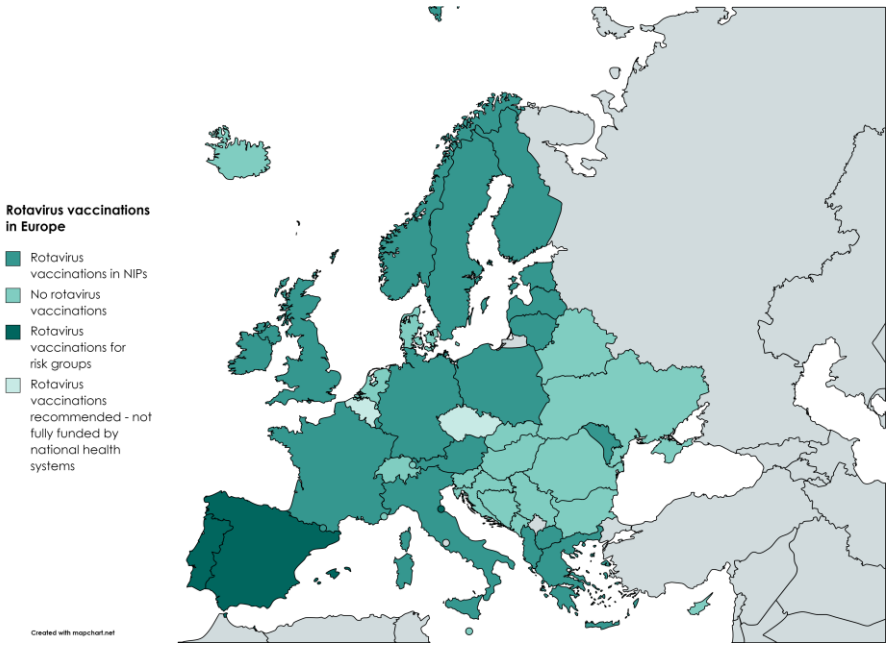

Figure 2. *Overview of rotavirus vaccinations in Europe.*
NIP: National immunisation programme.
Data sources: ECDC, WHO, NHS.

Rotavirus acute gastroenteritis is a vaccine-preventable disease. The World Health Organization has since 2009 recommended including rotavirus vaccinations in all vaccination programmes.[49] The global rotavirus vaccine coverage was estimated at 51% at the end of 2022,[7] whereas the vaccine coverage in Europe was estimated at 31%.[50] Rotavirus vaccinations have been introduced in the NIPs in 16/30 EU/EEA countries.[51] Figure 2 shows the countries in Europe that have implemented rotavirus vaccinations.[51-54]

WHO has prequalified four rotavirus vaccines,[55] and two vaccines have been licenced by the European Medicines Agency and the U.S. Food and Drug Administration: the monovalent Rotarix® against the G1P[8] strain, and the pentavalent bovine-human vaccine RotaTeq®, composed of G1, G2, G3, G4 and P[8] strains.[56-59] The two vaccines are made with a live attenuated

virus and are given orally in the first year of life, in two- and three-dose schedules, respectively. Both vaccines are safe and effective.[56,60,61] Side effects from the vaccines include diarrhoea, vomiting, fever, irritability and flatulence.[57,58]

A considerable reduction in diagnosed rotavirus infections, and rotavirus-associated visits to emergency departments and hospital admissions has been observed in countries where rotavirus vaccinations have been introduced.[35,41,62-64] In a recent study from Stockholm, rotavirus vaccinations led to a 78% decrease in admissions due to rotavirus and a 55% reduction in all-cause gastroenteritis admissions in children.[35] Cost-effectiveness analyses from high-income countries have shown the rotavirus vaccines to be cost-effective and even cost-saving.[65-69] The possible cost-effectiveness of implementing rotavirus vaccinations in Iceland was assessed in Study I.[42]

In a meta-analysis, the vaccine effectiveness (VE) of Rotarix® and RotaTeq® was estimated at 84-87% against laboratory-confirmed rotavirus infections for children under the age of two years in countries with low childhood mortality.[70] The VE against rotavirus infections needing medical care and admission has been estimated at 68% and 80%, respectively, for children aged 6-29 months.[64] In a meta-analysis, the VE of Rotarix® against homotypic strains was estimated at 90% and around 80% for fully or partially heterotypic strains.[71] For RotaTeq®, the VE was estimated to be 88% for homotypic strains and 82% for partially heterotypic strains.[71] The VE is lower in low-income countries and countries with higher childhood mortality for reasons that are not fully understood,[70,71] but malnutrition is hypothesised to play a part.[72,73] VE is higher in the first year after vaccination than in the second-year post-vaccination.[64] The number needed to vaccinate to prevent one rotavirus hospitalisation is estimated to be 442.[35]

Breakthrough infections occur in vaccinated children, but they are less severe than rotavirus infections in unvaccinated children and are less likely to require hospital admissions.[64] Parents of vaccinated children with breakthrough infections take fewer days off from work than parents of unvaccinated children with rotavirus infections.[64] Whether the rotavirus vaccinations affect seizure incidence remains disputed.[74-78]

In addition to the direct effects of the rotavirus vaccinations for vaccinated children, indications of herd protection of the vaccinations have also been noted.[41,62,79] For instance, a 50% reduction in laboratory-confirmed rotavirus infections was observed in individuals $\geq$5 years in England and Wales in the

first year following the introduction of rotavirus vaccinations.[62] However, not all studies show the same results regarding herd effects.[80]

The main concern of rotavirus vaccinations has been the possible increased risk of intussusception following vaccination, but studies are not unanimous regarding the intussusception risk of rotavirus vaccinations. One study showed an increase in intussusception hospitalisations in the weeks following immunisation in the age group 8-11 weeks, from 11.4 per 100,000 children in the pre-vaccination era to 16.7-22.9 per 100,000 children in the post-vaccination era.[81] In another study, a 6-10-fold increased incidence of intussusception was observed for both RotaTeq® and Rotarix® within the first seven days following the first dose of the vaccine. The risk decreased after that but was still increased for vaccine recipients for the first 21 days after vaccination.[82] Two meta-analyses did not show an increased risk of intussusception with rotavirus vaccinations.[17,83] Neither did a post-marketing study conducted in India on the WHO-prequalified vaccine Rotavac, a three-dose oral rotavirus vaccine.[84] Disease severity is also important in the risk assessment of the rotavirus vaccination. In one study, children with vaccine-associated intussusception were younger than those with non-vaccine-associated intussusception, but after adjusting for age, no association between intussusception severity and rotavirus vaccinations was observed.[85] If there is a slight increase in intussusception risk, it is estimated to be clearly outweighed by the decrease in disease burden from rotavirus infections.[81,82,86]

1.2.5 Rotavirus – summary

Rotavirus is one of the major pathogens in AGE and causes a significant disease burden.[32-35,39-42] Rotavirus vaccinations have not been introduced in the Icelandic immunisation schedule, despite the WHO recommending rotavirus vaccinations in all immunisation programmes.[49,87] The vaccines have shown enormous benefits in reducing rotavirus infections, ED visits and hospital admissions.[35,41,62-64] Rotavirus vaccinations are safe[56,60,61] and have been assessed as cost-effective, or even cost-saving, in other high-income countries.[65-69,88] In Study I, the disease burden of acute gastroenteritis and rotavirus infections leading to emergency department visits was evaluated, as well as the cost-effectiveness of rotavirus vaccinations in the Icelandic immunisation programme.

1.3 *Neisseria meningitidis*

1.3.1 The meningococcus

The meningococcus was first described as *Diplococcus intracellularis meningitidis* in a paper by Anton Weichselbaum, published in 1887 after he isolated the bacteria from the cerebrospinal fluid of patients suffering from meningitis.[89] In the more than 130 years since its identification, effective treatments and vaccines have been developed, but invasive meningococcal disease (IMD) remains a devastating disease with a high mortality[90-92] and morbidity in survivors.[93-95]

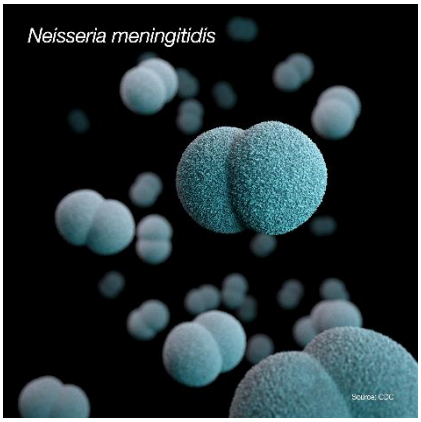

Figure 3. *Neisseria meningitidis.* An image based on electron microscopic imaging. Source: CDC. https://www.cdc.gov/meningococcal/about/photos.html

Neisseria meningitidis is a gram-negative, aerobic, oxidase-positive diplococcus (Figure 3). It typically has a coffee-bean shape on microscopy.[96] The meningococcal colonies are round, 1-5 mm in diameter and have a colourless or grey appearance when cultured on agar plates.[96]

N. meningitidis has a cytoplasmic membrane, a periplasmic membrane, and an outer membrane. The outer membrane consists of phospholipids, lipooligosaccharides and various outer membrane proteins (PorA, PorB, Opc, Opa, fHbp, NadA, NHBA, FetA).[96] *Neisseria meningitidis* may have a polysaccharide capsule, which is the main virulence factor and the basis for the meningococcal capsular group classification.[96,97]

The polysaccharide capsule is determined by the *cps* genomic region.[98] The *cps* genomic region can contain different genes corresponding to different capsular groups; *csb* corresponds to capsular group B, *csc* to capsular group C, *csw* to capsular group W and *csy* to capsular group Y.[98] The *cps* region can contain an intergenic region, termed capsule null locus (*cnl*), and those strains lack the polysaccharide capsule.[99]

Thirteen different capsular groups have been identified: A, B, C, D, E, H, I, K, L, W, X, Y and Z. Invasive meningococcal disease is most often caused by six capsular groups: A, B, C, W, X and Y.[98,100] Strains that lack a capsule, either because they lack or do not express capsular genes, or have a *cnl*, are

referred to as non-groupable meningococci.[101] Non-groupable strains can cause invasive infections, although they are rare.[102,103]

The capsular expression can be switched on and off by phase variation.[101,104-106] Phase variation involves reversible gene mutations, allowing the gene expression to be turned on or off.[107] The phase variation is believed to confer adaptability; the capsular off-switching may be beneficial for colonisation, while the expression of the capsule is important for the bacteria in invasive disease.[106,107] Furthermore, meningococci can undergo capsular switching by transferring capsular genes between strains, resulting in very closely related strains with different capsular groups. Capsular switching may promote an escape from immunity that has developed due to colonisation or vaccination.[108-110]

Different methods can be used to group meningococci. Serogrouping is generally done by using slide agglutination assays, whereas genogrouping is based on testing on genetic material from the bacteria, such as polymerase chain reaction (PCR) or whole genome sequencing (WGS).[111] Multilocus sequence typing (MLST), which compares internal fragments of housekeeping genes, can be used to classify meningococci to sequence types (STs). Meningococci are grouped into clonal complexes (CCs) based on the sequence types.[112]

Since the first description of meningococci in 1887, immense knowledge has been gained about meningococcal characteristics, colonisation, and mechanisms of invasion. However, despite over a century's worth of studies, many aspects of the complex relationship between the bacteria and its host remain to be explained.

1.3.2 Asymptomatic meningococcal colonisation and transmission

Asymptomatic meningococcal colonisation is common, although invasive disease is rare.[113-116] The carriage rates are low in childhood but increase with age, reach a peak during adolescence or young adulthood, and then gradually decrease with increasing age.[117,118] Due to this age distribution, most colonisation studies have focused on adolescents and young adults. Studies have shown carriage prevalence ranging from 2.7-59.1% in both vaccinated and unvaccinated cohorts.[113,114,119-133] Non-groupable meningococci are the most common meningococci in asymptomatic colonisation,[114,124] of which many have a *cnl.*[99,124,134]

Meningococci are transmitted with saliva, respiratory secretions and droplets.[106] Social behaviours, such as kissing, attending pubs/clubs and smoking, have been identified as risk factors for meningococcal colonisation,[114,119,121,124,125,128,129] as well as current or recent upper respiratory tract infections.[128] Meningococci have been shown to survive outside the host for up to eight days, underlining the possible transmission of meningococci by contaminated surfaces, such as drinking glasses and bottles.[135-137] As mentioned before, carriage prevalence peaks in adolescence or young adulthood.[118,119,124] In some studies, age has not remained a risk factor after adjusting for social factors, suggesting that social factors are the main drivers of colonisation among adolescents and young adults.[119,124]

Meningococcal carriage is frequently persistent, but the duration of follow-up varies between studies.[122,123,130,138-140] The mean duration of carriage has been estimated to be 11.7 months in children aged 3-14 years.[141] Studies have reported more than half of participants remaining colonised for at least three months.[123,130] In a study of military recruits, a third of participants were intermittent carriers of meningococci.[123] Interestingly, studies have shown a large proportion of non-carriers (50-70%) to remain non-carriers for the duration of the follow-up.[123,138]

Despite how common asymptomatic meningococcal carriage is, invasive infections are relatively uncommon.[113-115] Meningococcal colonisation induces an antibody response against meningococci, which develops within days after acquiring the bacteria.[142-144] Invasive disease generally occurs shortly after the acquisition, before a significant antibody response has developed.[142] Carriage is presumed to be the prerequisite for invasive disease.[145] However, high carriage rates in populations with a low incidence of disease are described,[117] as well as a high disease burden but low colonisation prevalence.[146] This suggests that other factors are also important in the development of invasive disease.

1.3.3 Invasive meningococcal disease

Six capsular groups of meningococci (A, B, C, X, W and Y) cause the majority of invasive meningococcal disease.[100] In general, invasive disease occurs shortly after the acquisition of *N. meningitidis,* before the development of a significant antibody response.[142]

1.3.3.1 Clinical presentation

Invasive meningococcal disease (IMD) typically presents with meningitis, septicaemia or both.[91,95,147] Atypical presentations, such as pneumonia, endocarditis, septic arthritis, and epiglottitis, are less frequent.[91,148] The clinical presentation varies with age and the capsular group causing IMD.[91,148] Atypical presentations are more common among older adults and those infected with capsular groups W and Y.[91,94,148-150] MenW has furthermore been reported to present with acute gastrointestinal symptoms, such as abdominal pain, nausea, vomiting and diarrhoea.[149,150]

Most cases of IMD occur in people without known risk factors, such as asplenia or complement deficiency,[91,151] although respiratory tract infections have been reported to precede invasive meningococcal disease in many cases.[94,152] IMD progresses rapidly, but initial symptoms are unspecific, such as fever, headache, nausea, and loss of appetite.[147] Abnormal skin colour, cold hands and feet, and leg pain are signs of early meningococcal disease in children and adolescents that usually present within the first 12 hours after disease onset.[147] Other signs and symptoms of IMD include altered mental status and meningism.[89,147] The characteristic haemorrhagic rash is apparent in 40-70% of cases among children.[93,147] Most deaths from IMD occur within one day of diagnosis of IMD.[90] The overall pooled case fatality rate (CFR) of meningococcal infections was estimated at 8.3% (95% CI 7.5-9.1%) in a meta-analysis,[92] with different capsular groups having different CFRs. MenW and MenC had the highest pooled CFRs, 12.8% (95% CI 10.7-15.0%) and 12.0% (95% CI 10.5-13.5%), respectively. MenB had lower CFRs than the other capsular groups, with a pooled CFR of 6.9% (95% CI 6.0-7.8%).[92] In an Icelandic study of IMD in a 30-year period, the 30-day CFR was 7.9%.[153] Age and clinical presentation affect CFR.[92,154] CFR generally increases with age, except for infants who have a higher CFR than older children.[90,92,154] It is also repeatedly reported that meningococcal sepsis carries higher CFRs than meningococcal meningitis.[91,147,154,155]

For survivors, IMD carries significant morbidity, with a large proportion (25-60%[93,95,151,156]) of patients having sequelae months to years after the infection. Sequelae include skin scarring, impaired hearing, visual defects, renal failure, amputations, disturbed postural control, and cognitive impairment.[93-95,156-159] The prevalence of sequelae does not differ between capsular groups causing IMD.[93,95]

The treatment for IMD consists of antibiotics, as well as supportive treatment for organ dysfunction or complications. IMD is usually treated with

benzylpenicillin or third-generation cephlosporins.[160,161] Antimicrobial chemoprophylaxis is recommended for close contacts (e.g. household members, students in the same dormitory and boyfriends/girlfriends) of IMD patients, but guidelines vary on who should be eligible for chemoprophylaxis following contact with patients with IMD.[160,162] Chemoprophylaxis should be given as soon as possible, preferably within 24 hours.[162] Ciprofloxacin is the recommended antibiotic for prophylaxis, and rifampicin is the alternative choice.[160,163] In addition to antibiotic prophylaxis, meningococcal vaccinations against the infecting capsular group are often offered to close contacts if they have not been vaccinated against the relevant capsular group in the past 12 months.[160]

N. meningitidis is mostly susceptible to the main antibiotics used for the treatment of IMD and chemoprophylaxis for close contacts.[164,165] Intermediate susceptibility to penicillin and ampicillin is relatively frequent (in up to 42.5% of strains tested[164-166]), and rates have increased over the past twenty years,[164,165] but resistance to penicillin, ampicillin, third-generation cephalosporins and rifampicin is rare.[164-166] However, penicillin- and ciprofloxacin-resistant β-lactamase producing MenY strains have emerged.[167]

1.3.3.2 Epidemiology

Most cases of IMD are sporadic but can occur in clusters or outbreaks.[168-172] Clusters are often comprised of two to three cases,[168] and most secondary cases occur within seven days of the primary case.[168] The incidence of IMD is highest among infants and young children with a smaller second peak in adolescence and young adulthood.[90,91,95,115,116,173] Death rates are, however, highest among older adults.[90,155]

Seasonality is observed for IMD, with most infections occurring in the winter[116,174] when viral respiratory tract infections are common. The number of IMD cases has been shown to increase following surges in influenza activity, supporting the longstanding hypothesis that viral respiratory tract infections are a risk factor for subsequent IMD.[174,175]

IMD epidemiology shows temporal and geographical variations.[176] In Europe, the overall incidence of IMD has decreased in the past years, from 0.95/100,000 people in 2008 to 0.62/100,000 people in 2017.[115] Figure 4 shows the notification rates (N/100,000 people) in Europe in 2019, when the notification rate for Europe in general was 0.57/100,000 people. The notification rates were highest in Ireland (1.37/100,000) and Lithuania (1.15/100,000), but lowest in Iceland, with no cases of IMD.[173]

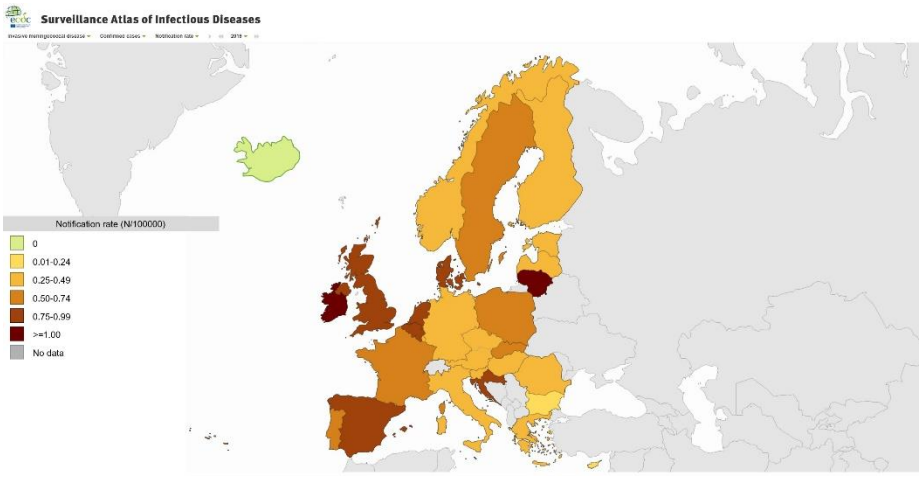

Figure 4. *Notification rates of confirmed cases of invasive meningococcal disease in Europe in 2019.*
The figure shows the inter-country variation in notification rates of invasive meningococcal disease in Europe in 2019. Iceland had the lowest notification rate, with no infections, but the highest notification rates were in Ireland and Lithuania. Source: ECDC - Dataset provided by ECDC based on data provided by WHO and Ministries of Health from the affected countries. The figure was adjusted from the ECDC Surveillance Atlas of Infectious Diseases.

MenB and MenC used to be the most common capsular groups causing IMD in Europe, but with the introduction of MenC vaccines, the incidence of MenC IMD decreased dramatically.[177] MenB has since been the prevailing capsular group in Europe,[115,116,178] but the incidence has been declining and was 0.3 cases per 100,000 individuals in 2017.[115] However, the incidence of IMD due to capsular groups W and Y has increased in the past years, [115,116,154,179-181] from 0.02 and 0.03 cases per 100,000 people in 2008 to 0.10 and 0.07 cases per 100,000 people in 2017, respectively.[115] The increase in MenW IMD was especially pronounced in the UK and the Netherlands,[179,181] with most isolates belonging to the hypervirulent clonal complex 11.[181] It led to changes in the NIP in both countries, with the Netherlands replacing toddler MenC vaccinations with MenACWY vaccinations and adding a quadrivalent MenACWY vaccine for 13-14-year-olds in the schedule.[182] In the UK, the adolescent meningococcal vaccinations were changed from a MenC vaccine to a MenACWY vaccine.[183]

In Iceland, MenB was the most common meningococcal capsular group causing IMD in 1975-2004, causing 48.2% of all cases of IMD.[153] MenC was the second most common, causing 28.5% of all cases, followed by MenA

(3.7%, 20 cases).[153] In the mid-1990s, the incidence of MenC increased substantially, and it became the dominant capsular group in IMD in Iceland, while the incidence of MenB decreased.[153] Vaccinations against MenC were added to the Icelandic immunisation schedule in 2002 with a catch-up campaign for ≤19 year-olds.[184,185] The vaccinations led to a significant reduction in the incidence of MenC IMD and an overall decrease in IMD incidence[186,187] (Figure 5[173]). The increase in the incidence of MenY and MenW IMD, which has been observed in some European countries[115,154] has not been observed in Iceland.

Interestingly, Iceland went from being one of the countries in Europe with the highest incidence of IMD to having one of the lowest incidences following the MenC vaccinations.[173] In the 20-year period 2003-2022, only seven cases of invasive MenC disease were diagnosed in Iceland, the last in 2007. Of the seven MenC cases, one occurred in a child. After the introduction of the MenC vaccinations, MenB has been the prevailing capsular group, with 0-6 cases diagnosed annually. No cases of IMD were diagnosed in Iceland in 2018-2021 (unpublished data from the Department of Clinical Microbiology at Landspitali University Hospital, courtesy of Helga Erlendsdóttir).

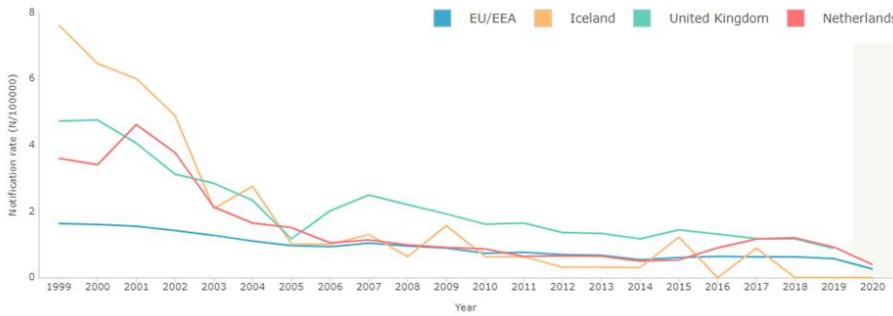

Figure 5. *Notification rate of invasive meningococcal disease in the EU/EEA, Iceland, the United Kingdom and the Netherlands from 1999 to 2020.*
The figure shows the declining notification rate (N/100,000 inhabitants) of invasive meningococcal disease in the EU/EEA (blue line), Iceland (orange line), the UK (green line) and the Netherlands (red line) in the years 1999-2020. Source: ECDC - Dataset provided by ECDC based on data provided by WHO and Ministries of Health from the affected countries.

1.3.4 Meningococcal vaccinations

Meningococcal vaccine development started in the early twentieth century, some years after the isolation of the meningococcus, but the first polysaccharide vaccines against meningococci were licenced in the 1970s.[188] Polysaccharide protein conjugate vaccines were subsequently developed, providing better protection for young children and a longer duration of protection.[176,189] Polysaccharide protein conjugate vaccines are available against four meningococcal capsular groups; A, C, W and Y.[189] A pentavalent polysaccharide protein conjugate vaccine with an X component (ACWXY) has undergone trials, which have shown the vaccine to be immunogenic and safe.[190,191]

Development of a vaccine against MenB proved more difficult than vaccines against capsular groups A, C, W and Y. The capsular polysaccharide of MenB is similar to human polysialic acid found in many glycoproteins, for example, in neural cell adhesion molecules (NCAM). It is consequently not very immunogenic.[97] Two protein-based vaccines against MenB are currently available, a four-component vaccine (4CMenB, Bexsero®, GSK) and an fHbp vaccine (Trumenba®, Pfizer).[192,193] The 4CMenB vaccine comprises three surface proteins (NadA, NHBA, fHbp) and an outer membrane vesicle (OMV) from the New Zealand MenB outbreak strain.[194,195] The fHbp vaccine consists of the two subfamilies of fHbp in equal amounts.[196] Pentavalent MenABCWY vaccines, using a combination of licenced MenACWY and licenced MenB vaccines, have been under development for a while, but none have been licenced yet.[197,198] Results from recent phase 3 trials including 10-25-year-old individuals indicate that pentavalent MenABCWY vaccines are non-inferior to vaccinations with quadrivalent MenACWY vaccines and MenB vaccines.[199,200]

In addition to protection against MenB, the 4CMenB vaccine has been shown to elicit bactericidal antibodies against MenC, MenY and MenW strains.[201-205] Interestingly, there are also indications of protective effects of MenB vaccinations against *Neisseria gonorrhoea.*[206-208] Anti-OMV and anti-NHBA antibodies, elicited by 4CMenB, recognise gonococcal antigens[207] and might provide cross-protective immunity to *N. gonorrhoea* to some extent.[206,208]

Meningococcal vaccines provide direct protection against invasive meningococcal disease, but their effect on carriage and bacterial spread varies. Vaccinations with monovalent MenC vaccines have led to a significant reduction in MenC carriage, with an estimated 90% VE against MenC

carriage.[209-211] Conflicting results have been published on the effects of quadrivalent MenACWY vaccines on the carriage of the vaccine capsular groups.[131,146,210,212] In a meta-analysis, MenACWY vaccinations were not found to protect against meningococcal colonisation.[210] In contrast, a large study from the UK revealed sustained low carriage of MenC and decreased carriage of MenY and MenW among adolescents and young adults after the introduction of MenACWY vaccines.[146] The authors state that the meta-analysis included studies conducted in low-carriage settings or too shortly after the MenACWY vaccination implementation, impacting the results.[146] The MenB vaccine does not reduce the carriage of MenB nor other capsular groups,[128,210] nor does it reduce the density of meningococcal carriage, capsulated or uncapsulated.[130]

Vaccinations against meningococci were introduced in Iceland in 2002. A monovalent polysaccharide protein conjugate vaccine against MenC (NeisVac-C®, Baxter) was added to the routine childhood vaccinations at six and eight months of age and there was a catch-up campaign for ≤19-year-olds.[184,185] The uptake of meningococcal vaccinations in the childhood immunisations has been 90-96% for the past ten years.[185,213-221]

Meningococcal vaccinations are important public health interventions, both as a preventive measure in routine vaccinations and as a part of outbreak management together with post-exposure chemoprophylaxis.[171,222-224] The success of meningococcal vaccinations is undisputed, with dramatic reductions in IMD incidence after the introduction of vaccinations, reducing both mortality and morbidity from a devastating disease.[223,224]

1.3.5 *Neisseria meningitidis* – summary

Neisseria meningitidis is a commensal organism in humans that can cause invasive disease, with high mortality and morbidity despite antibiotic treatment.[91-93,124] Most invasive infections are caused by six capsular groups (A, B, C, W, X and Y), and vaccines are licenced against five of those (A, B, C, W and Y).[100] Capsular group B is the predominant capsular group in Europe,[115,116] whereas both IMD incidence and carriage of MenC decreased dramatically after the implementation of MenC vaccinations.[209,224] The incidence of MenY and MenW IMD increased substantially in Europe in the past years, although the overall incidence of IMD decreased.[115,116,154,179] The increase in MenY and MenW infections led some countries to add MenACWY to their national immunisation programmes or replace MenC vaccinations with MenACWY vaccinations.[182,183] Since 2015, MenB vaccinations have also been added to NIPs in some countries.[225] Colonisation precedes invasive

disease; thus, knowledge of meningococcal carriage and invasive disease epidemiology is important to guide public health authorities on vaccination strategies. In Study II, the prevalence of meningococcal colonisation was assessed among children, adolescents, and young adults in Iceland, as well as the prevailing capsular groups, the duration of carriage and the relationship between strains isolated from the same carrier over time.

1.4 Influenza

1.4.1 The influenza virus

The influenza virus is an enveloped negative-sense single-stranded RNA virus that belongs to the family Orthomyxoviridae. It has a segmented genome consisting of seven or eight segments.[226,227] These segments encode proteins needed for cell entry, replication and spread. The surface glycoproteins haemagglutinin (HA) and neuraminidase (NA) are the main targets of neutralising antibodies against influenza, whether after vaccination or natural infection. Haemagglutinin binds to receptors on cell surfaces containing sialic acid and is responsible for the entry of the influenza virus into the cell. Neuraminidase facilitates releasing of influenza virus particles bound to non-functional receptors, promoting the virus's spread.[226]

Four influenza viruses are known, influenza viruses A, B, C and D. Influenza viruses A and B cause the vast majority of illness in humans.[226] Influenza C causes upper respiratory tract infections and influenza-like illness (ILI), mostly in young children.[228,229] Influenza A infects, apart from humans, a wide range of animals, such as swine, poultry and wild migratory birds, while influenza B and C are human pathogens.[226] In contrast, cases of influenza D have not been reported in humans, but it infects cows and pigs.[226]

Influenza A has 16 known subtypes of HA (H1-H16) and nine of NA (N1-N9).[226] Influenza A is named after its haemagglutinin and neuraminidase subtypes, for example H1N1. Influenza B does not have subtypes but splits into two lineages, B/Victoria/2/1987-like and B/Yamagata/16/1988-like.[226]

Accumulation of mutations in HA and NA, termed antigenic drift, leads to evasion of the influenza virus to pre-existing immunity from previous influenza vaccinations and infections.[226] It is the reason for the need for annual influenza vaccinations. Antigenic shift refers to the reassortment of genetic segments, which can happen when two viruses of the same type infect the same cell simultaneously. The antigenic shift usually occurs in animals, often wild aquatic birds. The virus can then be transmitted to

humans, either directly or through transmission from other animals. If human-to-human transmission occurs, the virus has the potential to cause a pandemic.[226]

1.4.2 Influenza infections

Influenza spreads between humans with droplets, aerosol and contact.[230] The median incubation times of influenza A and influenza B are estimated to be 1.4 days and 0.6 days, respectively.[231] Influenza often presents with abrupt onset of symptoms, which include cough, nasal congestion or runny nose, sore throat, fever, chills, myalgia, fatigue and malaise, nausea and vomiting.[226,232,233] The symptoms are usually most prominent at the beginning of the illness and gradually decline over 6-8 days.[233] The treatment of influenza infections is mainly supportive, although two classes of antivirals are available against influenza; NA inhibitors, such as oseltamivir, and adamantanes that target the M2 ion channel of the influenza A viruses.[226]

Seasonal influenza epidemics occur annually, usually during winter, when low humidity and cold temperatures facilitate spread.[226,234-236] Pandemic outbreaks occur every 10-50 years.[226] During pandemics, the virus spreads rapidly due to limited pre-existing immunity. The pandemic strains subsequently become the circulating strains in seasonal epidemics.[226] The last influenza pandemic occurred in 2009, with influenza A/H1N1.[226]

Influenza infections are common.[237,238] It is estimated that annually around 10% of unvaccinated adults and 20% of unvaccinated children <18 years old are infected with influenza, about half of them with a symptomatic infection.[237] The burden of influenza is, therefore, substantial.[238] For children, the burden of influenza is high.[239,240] With the high incidence of influenza and influenza-like illness (ILI) in the age group[237] comes a significant number of visits to primary health care and outpatient clinics.[241] Complications from influenza infections, such as secondary bacterial infections, are common among children, especially younger children and those with co-morbidities, and frequently lead to antibiotic prescriptions.[238,241-243] Children, particularly infants under the age of one, are among the groups most likely to require hospital admissions due to influenza infections.[239-242,244-246] Most children hospitalised due to influenza are previously healthy,[245,246] but children with underlying diseases have an increased risk of severe influenza infections.[239,245-249] Even though the burden of influenza is high in children, the overall mortality is low.[239-241,250,251]

1.4.3 Influenza vaccines

Inactivated vaccines are available against influenza. The inactivated influenza vaccines are mostly manufactured using chicken eggs. The process is rather cumbersome and takes several months.[226] A prediction is made by a group of experts from WHO on which strains are likely to be circulating the following influenza season. The eggs are co-infected with the predicted circulating strain and a high-growth strain, influenza A/Puerto Rico/8/1934 (PR8), so reassortment can take place. Vaccine strains are subsequently selected to have haemagglutinin and neuraminidase from the predicted circulating strain but other genome segments from the high-growth strain.[226] Eggs are infected with the selected strains and used for amplification.[226] This way of manufacturing influenza vaccines is not without faults. It involves predicting the antigenic drift that will occur and making a vaccine based on a prediction of what strains are likely to be circulating in six months. Therefore, the vaccine's effectiveness is often variable due to a mismatch of the vaccine and the circulating strains.

Live-attenuated influenza vaccines (LAIV) are also available.[226,252] They are produced using genetic reassortment, with haemagglutinin and neuraminidase encoding gene segments from a wild-type influenza virus and the other segments from a master virus.[253] The master virus is attenuated, cold-adapted and temperature sensitive so that the virus can replicate in the nasal mucosa but no other sites in the respiratory tract.[252,254] The LAIVs are administered intranasally. In Europe, the LAIVs are only licenced for children aged 2-18 years.[252] They are not licenced for children under the age of two years because of their association with wheezing in young children.[255,256] They are not contraindicated in children and adolescents with asthma, treated with inhaled corticosteroids or low-dose systemic steroids, but should not be used in those with severe asthma.[252] LAIVs are, like other live attenuated vaccines, contraindicated in people with immunodeficiency or on immunosuppressive therapies.[252]

The vaccine effectiveness of influenza vaccines varies by influenza season, the influenza vaccine strains, and age.[257,258] In meta-analyses, the reported VE from individual studies ranged from -85% to 93%.[257,258] The VE has been reported to be considerably lower for influenza A/H3N2 than A/H1N1 and the B influenza strains.[257,258] The VE is generally highest in children and decreases with age.[258]

mRNA vaccines against influenza have been under development for some years. A multivalent mRNA influenza vaccine against all known influenza

viruses has been developed. Testing in mice and ferrets has shown good protection, but the vaccine has yet to undergo human trials.[16]

1.4.3.1 Evaluation of the immune response to influenza vaccinations

There are several ways to evaluate the immune response to influenza vaccinations.[259] The most common is the haemagglutination inhibition (HAI) assay, which measures the concentration of antibodies needed to inhibit the binding of the viral haemagglutinin to erythrocytes, thereby inhibiting haemagglutination.[260] The titres are presented as the highest dilution that inhibits the haemagglutination. Microneutralization (MN) assay is another method. It measures the concentration of antibodies needed to prevent the influenza virus from infecting eukaryotic cells *in vitro* and inhibiting the cytopathic effects of the virus.[260] The MN titres represent the dilution at which influenza-induced cytopathic effects are observed in less than half of the wells.[261] MN measures functional antibodies and may be more sensitive than the HAI,[262] but it is also more laborious, requires handling a live virus and is more difficult to standardise.[260,262] The cellular immune system also plays an important part in the protection against viruses, and T cell stimulation assays can be used to assess cell-mediated immunity.[261] They may give a better picture of the protection from influenza vaccines than the serological methods.[263]

1.4.4 Influenza and obesity

Obesity is prevalent among both adults and children/adolescents in Europe.[264,265] In 2011-2016, 6.3% of European children aged 2-13 were estimated to have obesity.[264] Icelandic data shows that 7.3% of 9-10-year-olds and 8.2% of 14-15-year-olds have obesity.[266]

Obesity emerged as an independent risk factor for more severe influenza infections, complications and death during the 2009 influenza A/H1N1 pandemic.[267,268] An association between obesity and more severe seasonal influenza has also been observed.[269,270] Studies do not show consistent results regarding the risk associated with obesity in children. Two studies reported that children with obesity are not more likely to get a symptomatic influenza infection than children that do not have obesity.[271,272] In contrast, another revealed an increased risk of influenza infections associated with obesity, especially abdominal obesity.[273] When infected with influenza, children with obesity miss more days from school than children without obesity,[271] which might imply more severe or prolonged symptoms. In a

meta-analysis, obesity was not a risk factor for hospitalisation.[274] Obesity has additionally been associated with an increased duration of viral shedding of influenza A among adults, although not the duration of influenza B virus shedding.[275] Children with obesity (5-17 years old) do not have a longer duration of shedding than children with normal weight.[275]

Due to the increased risk of severe disease associated with obesity, protection with vaccinations is of utmost importance. However, reduced vaccine responses have been associated with obesity. Obesity in adults has been associated with a poorer response to hepatitis B vaccinations.[276,277] Children with overweight and obesity were found to have lower levels of anti-tetanus titres after vaccination than children with normal weight.[278] The clinical importance of this difference is unknown since the anti-tetanus titres were adequate in both groups.[278] Studies in adults have not indicated poorer humoral responses to influenza vaccinations in individuals with obesity,[279-281] but obesity might affect the longevity of the response and the cellular immune response.[279,281]

Only a few studies have been done to assess the influenza vaccine responses in children with overweight and obesity.[282-285] None of these studies showed reduced vaccine responses in children with overweight and obesity compared to children with normal weight.[282-285] Three studies had a combined study group with children with overweight and obesity,[282-284] which might have affected the results. In an observational cohort study, the VE of influenza vaccination was estimated at 72.7% (95% CI 25.7-90.0%) in 5–13-year-old children with obesity, higher than in children with normal weight of the same age, although not statistically significant.[271] The study presented in this thesis is, to our knowledge, the first one to assess both the humoral and the cellular immune response in a study group comprised only of children with obesity, thereby adding to the knowledge of the effects of obesity on influenza vaccination responses.[286]

1.4.5 Influenza, pregnant women, and infants

Pregnancy has been identified as a risk factor for more severe influenza infections.[267] In the 2009 influenza A/H1N1 pandemic, pregnant women were more likely to be admitted to hospital and had an increased risk of death compared to non-pregnant women of childbearing age.[267] In two meta-analyses, influenza infections in pregnant women were associated with an increased risk of hospitalisation compared to non-pregnant women but not higher mortality nor increased risk of complications, although individual studies show varying results.[287,288]

Influenza vaccinations are globally recommended for pregnant women,[289,290] as a preventive measure both for the mother and the unborn child.[291] WHO recommended, in a 2012 position paper, that pregnant women should be in the highest priority for influenza vaccinations.[290] Influenza vaccinations in pregnancy have been shown to elicit seroprotective titres in most pregnant women.[292] Despite serologic evidence of seroprotection, vaccine effectiveness is estimated to be 44-50% against influenza infections in pregnant women[291,293] and 40% against influenza-associated hospitalisations.[294] Influenza vaccinations have, furthermore, been associated with reduced likelihood of preterm birth, low birth weight, and newborns being small for gestational age, although studies show inconsistent results.[295-299]

Influenza vaccinations in pregnancy (also known as maternal vaccinations) are the only available preventive measure against influenza in newborns, as influenza vaccines are not licenced for infants younger than six months of age. Influenza places a significant disease burden on infants,[239,241,244,300] and can be fatal.[248,251] Vaccinations during pregnancy have been shown to reduce laboratory-confirmed influenza cases in infants less than six months of age by 35-70%.[291,299,301-303] Furthermore, infants born to vaccinated mothers are less likely to need hospital admission should they get an influenza infection.[302,304]

Importantly, influenza vaccinations for pregnant women have repeatedly been shown to be safe for both the mother and the unborn child.[291,305,306] Despite the observed benefits, suboptimal influenza vaccine uptake (7.5-35%) in pregnant women is frequently reported.[295,296,302,307,308]

1.4.6 Influenza summary

The influenza virus causes seasonal epidemics with a substantial disease burden.[226] Obesity has been identified as a risk factor for more severe influenza infections in adults,[267-270] but whether obesity is associated with more severe disease in children is uncertain.[271-274] Pregnant women are at increased risk for severe influenza,[267,287] and infants are among the groups with the highest influenza disease burden.[239,241,244,248]

Protecting vulnerable groups against influenza is paramount, and influenza vaccines were the topics of studies III and IV presented in this thesis. In study III, the influenza vaccine responses in adolescents with obesity were compared to the vaccine responses in adolescents with normal weight to assess whether changes are needed to better protect adolescents

with obesity against influenza. In study IV, the influenza vaccine coverage in pregnant women was assessed over ten influenza seasons, as well as the burden of influenza in pregnant women and their infants.

1.5 Summary and introduction to the studies

Vaccinations are among the most important medical discoveries ever made and are the cornerstone of public health measures. Millions of lives are saved globally every year with immunisations.[3] Despite the enormous benefits of vaccinations, existing and emerging infectious diseases remain public health challenges. The local epidemiology of vaccine-preventable diseases is important when it comes to decision-making regarding the addition of new vaccines or changes to the national immunisation programme. Additionally, it is pivotal that healthcare providers are aware of the importance of protecting vulnerable groups against vaccine-preventable infectious diseases. In this thesis, potentials for improvements in the national immunisation programme in Iceland are explored, with a focus on the three pathogens covered in the introduction – rotavirus, *Neisseria meningitidis,* and influenza.

Rotavirus is the leading cause of AGE in young children. It causes a significant burden on children, families, healthcare systems and society.[32-35,39-42] Vaccines are available against rotavirus and are recommended by WHO,[87] but have not been introduced in Iceland. In study I, the burden of RVAGE in young children and the cost-effectiveness of rotavirus vaccinations in Iceland were assessed to answer the question whether rotavirus vaccinations should be added to the Icelandic national immunisation programme.

Neisseria meningitidis is a common human upper respiratory tract commensal that can cause serious infections, with a high case-fatality rate and sequelae in survivors.[92,95,114,122,124,131] Six capsular groups cause most IMD cases; vaccines are currently available against five.[100] Asymptomatic colonisation and carriage dynamics are important factors in meningococcal epidemiology that can help guide decisions on vaccination strategies. In study II, the meningococcal carriage and prevalence of the different capsular groups were assessed, as well as the duration of meningococcal carriage.

Influenza causes seasonal epidemics every year with a considerable disease burden.[237,240] Certain groups, such as infants, pregnant women and people with obesity, are at increased risk for serious influenza infections.[239,241,244,267,269,270,287,288,300] Influenza vaccinations are the major preventive measure against influenza infections and are especially important

to protect those at increased risk. Obesity affects the immune responses to some vaccinations.[276-278] In study III, the immune responses to influenza vaccinations were assessed in adolescents with obesity compared to adolescents with normal weight to determine if changes are needed to provide a better protection for a possible risk group. Influenza vaccinations are recommended in pregnancy to protect both the pregnant woman and her unborn child.[290] In study IV, the influenza vaccine uptake in pregnancy and the influenza disease burden on pregnant women and their infants was assessed over ten influenza seasons – to shed light on whether measures need to be taken to improve influenza vaccination coverage in pregnant women.

2 Aims

The aims of the thesis were to assess whether changes and improvements are needed to the Icelandic national immunisation programme regarding immunisations against rotavirus, *Neisseria meningitidis* and influenza. The aims of the studies comprising this thesis were as follows:

I. **Study I – The RICE study:** To assess the disease burden of acute gastroenteritis and rotavirus acute gastroenteritis in young children leading to emergency department visits in Iceland. To estimate the cost-effectiveness of including rotavirus vaccines in the Icelandic national immunisation programme.

II. **Study II – The MENICE study:** To determine the prevalence of asymptomatic meningococcal carriage among children, adolescents and young adults in Iceland, the dominant capsular groups, the duration of carriage and whether changes should be made from the current MenC vaccination schedule.

III. **Study III – The OFICE study:** To assess the immune response of adolescents with obesity to a tetravalent influenza vaccine, compared to adolescents with normal weight, to evaluate whether changes are needed in influenza immunisations for adolescents with obesity.

IV. **Study IV – The FluRisk study:** To assess the influenza vaccine uptake in pregnant women and the burden of influenza in pregnant women and their infants to identify whether initiatives are needed to improve influenza vaccine uptake in pregnancy.

3 Materials and methods

3.1 Study I – RICE: Burden of rotavirus disease in young children in Iceland

3.1.1 Study design and participants

All children <6 years of age presenting to the paediatric emergency department (ED) at the Children's Hospital Iceland with acute gastroenteritis (AGE) during the two-year period 1st January 2017 to 31st December 2018 were invited to participate. Acute gastroenteritis was defined as $\geq$ 3 loose stools and/or $\geq$ 2 vomiting within 24 hours, with an onset in the last 14 days. An informed consent form was signed by parents/legal guardians of the participants at enrolment. Information was collected on age, sex, daycare attendance, the duration of symptoms prior to the ED visit, Vesikari score[31] of the AGE episode and the treatment given in the ED (antiemetics, oral fluids, intravenous fluids). Stool samples for virological analysis were collected from participants. Anal skin swabs were done if stool samples were not attainable. Samples were also sent for bacterial culture if the clinical presentation or medical history suggested a possible bacterial infection.

Parents/guardians were contacted by telephone within one week of the ED visit and asked about the duration of illness, days missed from daycare, the number of days parents/guardians missed from work or school and how many household members were also infected. Furthermore, they were asked about their opinion on rotavirus vaccinations. If the child still had symptoms of AGE, the parents were phoned again two weeks after the ED visit.

Data on the number of ED visits for children <6 years of age with the diagnostic codes for AGE (ICD-10 codes A08 and A09) was retrieved from a hospital register.

3.1.2 Laboratory methods - virology

Stool samples were analysed at the Department of Virology at Landspitali University Hospital. Standard laboratory methods were used for DNA extraction and quantitative polymerase chain reaction (qPCR).[309] qPCR was done for rotavirus, norovirus, adenovirus, astrovirus, enterovirus and sapovirus. The qPCR for adenovirus was not specific for the enteropathogenic serogroups 40 and 41. Samples with quantification cycle

(Cq) values <37 were considered positive, and samples with Cq-values between 37 and 40 were defined as weakly positive.

3.1.3 Definitions

When comparing RVAGE to non-rotavirus AGE, all AGE episodes in which rotavirus was identified (either as a sole pathogen or as one of the identified pathogens) were defined as RVAGE. AGE episodes in which other pathogens or no pathogens were detected were defined as non-rotavirus AGE. Pathogens detected as weakly positive on qPCR were included if they were the only identified pathogen in the sample but excluded if other pathogens were identified in the sample. Visits to the ED on three or more consecutive days were defined as hospital admissions. Episodes with Vesikari scores $\geq$11 were defined as severe gastroenteritis, scores of 7-10 as moderately severe gastroenteritis and scores <7 as mild gastroenteritis.

3.1.4 Cost-effectiveness calculations and statistics

Costs were converted from Icelandic kronur (ISK) to Euros (€). The average exchange rate for 2018 was used, according to which 1€ equalled 127.73 ISK.[310]

Estimations of the cost of RVAGE included direct healthcare costs and indirect costs from loss of productivity during parental sick leave. Secondary cases in the home were not included in the cost analysis. Hospital cost data (cost of ED visits and admissions) was retrieved from a register at Landspitali University Hospital. Ten per cent of children <6 years of age were estimated to be infected with rotavirus annually. Five and a half birth cohorts were assumed to be susceptible each year (approximately 23,000 susceptible children[311]). Parents/guardians were assumed to miss three days of work for each primary infection (other studies report 2.5-7.5 missed days from work).[40,64,88,312] The value of each day lost from work was estimated at €367, based on the average salary of working people in Iceland, including wage-related expenses, retrieved from Statistics Iceland.[313]

The χ^2 test and Mann-Whitney *U* test were used for comparisons between groups. A *p*-value <0.05 was considered statistically significant. Statistical analyses were done using R (version 3.6.3).

3.2 Study II – MENICE: Meningococcal colonisation in children, adolescents, and young adults in Iceland

3.2.1 Study design and participants

A descriptive point-prevalence and longitudinal study on asymptomatic meningococcal colonisation was done in the greater Reykjavík capital area, Iceland. The study included three age groups: 1) 1–6-year-old children attending daycare centres (DCCs) recruited from 15 DCCs; 2) 15-16-year-old adolescents in the last year of lower secondary school, recruited from five secondary schools; and 3) 18–20-year-old college students recruited from three colleges. An informed consent form was signed by parents/legal guardians of participating children in DCCs, both parents/legal guardians and the participating adolescents, and by the participating young adults prior to sample collection. The participating DCCs and schools approved participation in the study.

Participants were recruited, and the first swabs were collected in March and April 2019. Carriers were invited to participate in the longitudinal arm of the study, with follow-up swabs at three-to-six-month intervals, until the first negative swab.

For adolescents and young adults, data on meningococcal vaccinations and filled prescriptions for antibiotics in the 30 days preceding the first swab was obtained from registers governed by the Directorate of Health, Iceland. Data on filled antibiotic prescriptions between swabs was collected for participants in the longitudinal arm of the study. Data on tonsillectomies was obtained from the Icelandic Health Insurance and Landspitali University Hospital. Data on meningococcal vaccinations, antibiotic use and tonsillectomies was only collected for adolescents and young adults. Parents of children attending DCCs filled out a questionnaire on antibiotic use on the day of sample collection and in the past 30 days.

3.2.2 Sampling and laboratory methods

3.2.2.1 Sample collection and culture

Nasopharyngeal swabs were collected from the 1-6-year-old participants, and oropharyngeal swabs, swabbing both tonsils/tonsillar beds and the posterior oropharynx, from adolescents and young adults. eSwab pins® (Copan, Brescia, Italy) were used for specimen collection, and swabs were immediately placed in 1 ml liquid Amies transport medium that comes with the pins. All swabs were collected by healthcare personnel. Samples were

transported to the microbiology laboratory at Landspitali University Hospital and plated within six hours of collection. Samples were plated on blood agar, chocolate agar and GC agar and subsequently incubated at 36°C in 5% carbon dioxide. The following day, plates were visually examined for growth of *Neisseria meningitidis.* Colonies suggestive of *N. meningitidis* were tested for oxidase reaction (MERCK), and if positive, a single colony was cultured on chocolate agar (in-house made). The growth was then tested with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker, Bremen, Germany) using the MBT Compass Library DB-7854. The swab pins in the transport medium and the meningococcal strains were frozen and stored at -80°C for later testing.

3.2.2.2 Molecular analysis

DNA was extracted from the Amies transport medium for qPCR. Frozen strains were re-cultured on chocolate agar, from which DNA was extracted for qPCR and whole genome sequencing (WGS). A MagNA Pure system (Roche Molecular System, Pleasanton, IL, USA) was used for DNA extraction.

Samples identified as positive for *N. meningitidis* by culture and MALDI-TOF MS were further analysed with qPCR to detect meningococcal DNA. qPCR was done on DNA extracted from the Amies transport medium (hereafter referred to as oropharyngeal swabs, OPS) and cultured strains. qPCR was also done on every tenth culture-negative sample collected at the first sampling time point. *metA* and *ctrA* were used as gene targets for meningococcal detection.[314,315] qPCR for determination of genogroups was done on the OPSs. The gene targets for genogroups A, B, C, W, and Y were *csaB, csb, csc,*[315] *csw*[179] and *csy*,[315] respectively. Samples with Cq-values $\leq$40 were considered positive. The overall bacterial abundance in the samples was assessed with 16S qPCR.[316] Using the results from the *metA* qPCR and 16S qPCR, the abundance of *N. meningitidis* relative to the overall bacterial abundance in the samples was calculated.

All groupable *N. meningitidis* strains that were viable for culture after freezing were subjected to WGS. Nextera DNA Flex Library Prep kit (Illumina, San Diego, CA, USA) was used for library preparation and paired-end sequenced (2x150 bp) on an Illumina NextSeq platform (Illumina, USA), following the manufacturer's protocols.

3.2.2.3 Biochemical testing

Thirty-four strains were tested with API NH® (bioMérieux, La Balme-les-Grottes, France), a biochemical test for fermentation and enzyme reactions; twelve strains that were identified as *N. meningitidis* with MALDI-TOF MS but had *metA* Cq-values ≥40 on the OPS and 22 randomly selected strains, identified as *N. meningitidis* with MALDI-TOF MS. Results from qPCR of the cultured strains were used as a reference. The researchers involved in the biochemical testing were blinded to the results from qPCR on the cultured strains while carrying out the biochemical testing to limit the risk of reading bias.

3.2.3 Data analysis and statistics

For analysis of the WGS data, the Juno-assembly v2.0.2 pipeline (GitHub - RIVM-bioinformatics/Juno_pipeline) was used for read quality analysis and *de novo* assembly. FastQC and FastP were used for read quality assessment and filtering and SPAdes was used to assemble genomes. They were curated with QUAST, CheckM and Bbtools. Isolates were typed with MLST v2.19.0 (GitHub - tseemann/mlst). *In silico* capsule typing was done, as has previously been described by others.[317] The core genome multilocus sequence typing (cgMLST) scheme on PubMLST was used for cgMLST.[318,319] A minimum spanning tree was constructed from the cgMLST data, using GrapeTree v2.1.[320] The data is available on PubMLST (isolate IDs 123755-123768).

The Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) Index[321] on PubMLST was used to assess the reactivity of the two licenced protein-based meningococcal B vaccines against the sequenced meningococcal carriage strains.

RedCap® was used for participant data registration and management.[322,323] Descriptive statistical analyses were done. The χ^2-test and Fisher's exact test were used to compare categorical variables. Mann-Whitney *U* test was used to compare numerical variables. Univariable and multivariable logistic regression analyses were done to assess risk factors for meningococcal carriage, using the variables age, sex, meningococcal vaccinations, antibiotic use in the past 30 days and tonsillectomies. A *p*-value of <0.05 was considered statistically significant. Statistical analysis was done in R (version 4.2.2).

3.2.4 Definitions

Samples were considered positive for meningococci if they were both culture positive and positive for *metA* on qPCR from the oropharyngeal swab or the cultured strain. In cases of culture-positive samples that were qPCR negative or unavailable for qPCR, they were considered positive for meningococci if the MALDI-TOF MS score was ≥2. Samples positive for both *metA* and *ctrA* were defined as encapsulated meningococci. Samples positive for *metA* only were defined as non-encapsulated meningococci (referred to as non-groupable (NG) meningococci hereafter). Prolonged carriers were defined as participants having two or more consecutive samples positive for *N. meningitidis.* Participants that had two consecutive samples taken, with the latter being negative, were defined as non-prolonged carriers.

According to the MenDeVAR[321] index, the definitions of reactivity are as follows: Exact match if there is ≥1 exact sequence match to a vaccine antigen variant; cross-reactive if ≥1 isolate antigen variant has been shown cross-reactive to a vaccine antigen variant in experimental studies; no reactivity if experimental studies have not shown cross-reactivity between the strain‘s antigen variants and the vaccine antigen variants; insufficient data if not enough data is available on the isolate‘s antigen variants or they have not been tested in experimental studies.[321]

3.3 Study III – OFICE: Influenza vaccine responses in adolescents with obesity

3.3.1 Study design and participants

The study group consisted of 30 adolescents, aged 12-18 years, with obesity and the control group of 30 adolescents of the same age with normal weight and without diseases that could affect immune function. Obesity was defined by age-adjusted body-mass index (BMI), according to international standards.[324] The study group was recruited through the Obesity treatment team at the Children’s Hospital in Reykjavik, Iceland and the control group through professional and personal contacts of the study team. All participants and their parents/legal guardians signed an informed consent form.

Participation in the study included two study visits in November and December 2020. In the first visit, venous blood samples were collected, body measurements were done, and participants were vaccinated with a tetravalent influenza vaccine. The second visit was four weeks later, with a maximal delay of 14 days. It included a collection of venous blood samples

and body measurements. The participants were compensated for their time participating in the study with a €70 gift voucher.

Body measurements included height, weight (wearing clothes estimated to weigh one kilogram), waist circumference and body fat percentage. TANITA MC-780 was used to weigh participants and measure the body fat percentage with bioelectrical impedance analysis (BIA). The calculated BMI was retrieved from the TANITA MC-780. Waist circumference was measured, as suggested by a WHO Expert Consultation report,[325] midway between the lower edge of the last palpable rib and the top of the iliac crest.

Participants were vaccinated with 0.5 ml of inactivated tetravalent influenza vaccine (VaxigripTetra®, Sanofi Pasteur MSD) with an intramuscular injection in the deltoid muscle using a conventional needle size. A trained healthcare professional administered all vaccines. The 2020/2021 influenza vaccine was composed of the following influenza strains: A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like virus (A/ Guangdong-Maonan/SWL1536/2019, CNIC-1909), A/Hong Kong/2671/2019 (H3N2)-like virus (A/Hong Kong/2671/2019,IVR-208), B/Washington/02/2019-like virus (wild type) and B/Phuket/3073/2013-like virus (wild type). The vaccinations were registered in the patient's electronic medical records. 28 ml of venous blood were collected at both study visits, 18 ml in heparin bottles and 5 ml in serum bottles.

3.3.2 Immune response

3.3.2.1 Haemagglutination inhibition (HAI) assay

A haemagglutination inhibition (HAI) assay was done to assess the humoral immune response.[326] It was done by the Public Health England (PHE) laboratory, Virus Reference Department, London, UK. Serum samples were stored at -80°C prior to analysis. HAI assay was done with antigens grown in-house at the PHE laboratory, representing the four influenza strains included in the vaccine. Three strains (Influenza H1N1pdm09 and both influenza B viruses) were grown in 10-day-old hen's eggs, while the influenza H3N2 strain was grown in tissue culture (MDCK cells). Tween/Diethyl-Ether was used to extract the influenza B antigens prior to analysis to increase assay sensitivity. Pre-validated control sera of human and ferret origin were used during all assays, and titres of these controls were used to monitor and validate assay performance.

Receptor Destroying Enzyme from *Vibrio cholerae* (RDE II, Denka Seiken) was used to treat sera prior to analysis, according to the

manufacturer's recommendation. Turkey red blood cells (RBCs) were also used to haemadsorb sera when necessary. Pre-treated sera were examined in duplicates by serial dilution (two-fold, starting at 1:10), and then each was incubated with four Haemagglutination Units (HAU) of antigen. Inhibition of agglutination was assessed with turkey RBCs for A/H1N1pdm09, and the influenza B strains and guinea pig RBCs for A/H3N2.

Analyses where titre duplicates for each serum-virus combination were not in concordance with each other (more than a 2-fold difference in titres), were repeated. The dilution series ranged from 10-1280. If the endpoint was not reached at 1280, endpoint titration was done, with the highest dilution at 20480. Laboratory personnel were blinded to the groups and pre-/post-vaccination status of the samples.

3.3.2.2 T cell stimulation assay

T cell stimulation assays were done to assess the cellular immune response. T cells were stimulated with the influenza vaccine strains. Solutions containing the following conjugated monoclonal antibodies were used to stain single-cell suspensions: CD3 BV510, CD4 BV421, CD8 BV605, INF-γ BV711, TNF Alexa Fluor® 700, IL-13 APC and IL-2 PE (BD Biosciences). In cases of insufficient cell numbers, the stimulations with viral strains were prioritised as follows: Negative control, positive control, A/H1N1, A/H3N2, B/Victoria and B/Yamagata. Flow cytometry was used to analyse samples. While carrying out the T cell stimulation assay, laboratory personnel were blinded to the samples' groups.

Isolation of peripheral blood mononuclear cells (PBMCs)

Blood was diluted at 1:1 with sterile phosphate-buffered saline (PBS, in-house made). Fifteen millilitres of Ficoll-paque (Histopaque, Sigma-Aldrich) and up to 35 ml of blood/PBS solution were added to a 50 ml falcon tube (Sarstedt). The tube was centrifuged for 30 minutes without a break at 400g and 20°C. Then the lymphocyte layer was collected. The cells were washed three times by adding 10 ml of cold PBS, centrifuging the tube at 300g for 10 minutes at 4°C and discarding the supernatant. Cells were resuspended in a 1-3 ml medium containing 50% newborn calf serum (NBCS, Gibco) and 50% RPMI-1640 (Gibco) and put on ice. The same amount of freezing medium (20% dimethyl sulfoxide (DMSO, Sigma-Aldrich), 20% NBCS, 60% RPMI-1640) was gently added dropwise to the cells, with mixing. Cells in freezing media were placed in cryotubes (Nunc; Sigma-Aldrich) and Mr. Frosty

(Thermo Fisher Scientific), stored overnight at -80°C before being transferred to liquid nitrogen storage until further use.

PBMCs thawing and stimulation

PBMCs were rapidly thawed and transferred to a sterile 15 ml tube containing pre-warmed thawing solution (PBS without Ca^{2+} Mg^{2+} ((Gibco Life Sciences) containing 2.5 mM EDTA (PanReac AppliChem) and 20 µg/mL DNAse I (Sigma)). Samples were washed twice with centrifugation at 311g for 10 minutes at room temperature (RT) and were then counted. PBMCs were resuspended in complete medium prepared as follows: RPMI-1640 with L-Glutamine (Gibco) combined with 1% foetal bovine serum (FBS, Euroclone), 1% Penicillin/Streptomycin 100X (Euroclone), 1% Na pyruvate 100X (Gibco), 1% Non-essential amino acids 100X (Gibco), 1 µg/ml anti-CD28 (Purified NA/LE Mouse Anti-Human CD28, BD Biosciences, Clone CD28.2) and 1 µg/ml anti-CD49d (BD Pharmingen™ Purified NA/LE Mouse Anti-Human CD49d, BD Biosciences). Cells were stimulated with one of the following stimuli at a specific final concentration pre-determined with an experimental setup: 1 µg/ml of Antigen A/HongKong/2671/2019 (20/132), 1 µg/ml of Antigen A/Guangdong-Maonan/SWL1536/2019 (19/312), 2 µg/ml of Antigen B/Phuket/3073/2013 (21/136) or 2 µg/ml of Antigen B/Washington/02/2019 (19/238). Cells were cultured in a 96-well round bottom plate for each condition in a complete medium and incubated for 16 hours at 37°C in a humidified atmosphere containing 5% CO_2. Basal PBMC cultures, incubated in complete medium were used as negative control. Positive control was prepared by incubating cells in a complete medium supplemented with Staphylococcal enterotoxin B (SEB) from *Staphylococcus aureus* (Sigma Aldrich) at the final concentration of 1 µg/ml.

Brefeldin A (BFA) (Sigma) was added to each well after two hours of incubation at 37°C in a humidified atmosphere containing 5% CO_2, with a 5 µg/ml concentration.

Flow cytometry analysis (Fluorescence-Activated Cell Sorting, FACS)

After a 16-hour incubation overnight, the plates containing PBMCs were centrifuged at RT at 699g for 4 minutes. The supernatant was then discarded. PBMCs were washed with 200 µl/well of PBS-2.5 mM EDTA and centrifuged at 699g for 4 minutes. Cell staining was done with Live/Dead staining (Thermo Fisher) (1:1000 in PBS). The cells were then incubated for 20 minutes at RT in the dark. Cells were washed twice in PBS-2.5 mM EDTA and centrifuged at RT at 699g for 4 minutes. Cells were permeabilised,

incubating with 1X BD cytofix/cytoperm for 20 minutes at 4°C in the dark. After that, they were washed twice with 1x perm/wash buffer in PBS-2.5mM EDTA + 1% bovine serum albumin (BSA) and centrifuged at 699g for 4 min at 4°C (+2/+8°C). To identify T cell subsets, the supernatant was removed and single-cell suspensions were stained with the appropriate combination of the following directly conjugated monoclonal antibodies (MoAb) diluted in perm/wash buffer diluted in PBS-2.5mM EDTA 1% BSA, containing 2% normal rabbit serum: CD3 BV510, CD4 BV421, CD8 BV605, INF-γ BV711, TNF Alexa Fluor® 700, IL-13 APC and IL-2 PE (BD Biosciences). The cells were incubated for 20 minutes at RT in the dark. Cells were washed twice with perm/wash buffer 1x, centrifuged at 699g for 4 minutes at RT and resuspended in PBS-2.5 mM EDTA. A FACS machine (BD LSR Fortessa, combined with a PC FACSDiva software 8.0.1 (BD Biosciences)) was used to analyse the samples. Dead cells were excluded from the analysis.

3.3.3 Definitions, data analysis and statistics

The required sample size was estimated to be 30 in each group, using power calculations to show a 25% difference in HAI with 80% power, assuming a 15% dropout.

Samples with undetectable HAI titre were given the arbitrary titre of five for geometric mean titre (GMT) calculations. GMTs were calculated for each pair of measurements for every participant. Seroconversion was defined as a four-fold increase in the titre from pre- to post-vaccination. Seroprotection was defined as a titre $\geq$40.

If the negative control yielded a higher response than the positive control in the T cell stimulation assay, the experiment was excluded from the analysis.

The Shapiro-Wilk test was used to assess the normality of data. Student's t-test, Mann-Whitney *U* test and Fisher's exact test were used for comparisons between groups. The Benjamini-Hochberg procedure was used to correct for multiple comparisons. A *p*-value of <0.05 was considered statistically significant. Data analysis was done in R (version 4.2.0).

3.4 Study IV – FluRisk: Influenza vaccine uptake and burden of influenza illness in pregnant women and their infants

3.4.1 Study design

The influenza vaccine uptake among pregnant women and the burden of influenza in pregnant women and their infants was studied in a retrospective study covering ten influenza seasons, 2010-2020. Pregnant women in Iceland are offered an ultrasound at 20 weeks of gestation, which most accept. The study cohort of pregnant women included women attending a 20-week ultrasound at Landspitali University Hospital in Reykjavik from August to April each influenza season. Pregnancies with 20-week ultrasounds scheduled in May, June and July were excluded, as antenatal appointments for those pregnancies likely did not coincide with the influenza vaccination periods. Infants born following the pregnancy included in the cohort of pregnant women were included in the cohort of infants. The Birth Register, a central national database of all births in Iceland, was used to identify infants to be included in the study.

Central national registers, governed by the Directorate of Health in Iceland, were utilised for data collection. National identification numbers were used for data collection and to combine the databases. Data on influenza vaccinations for pregnant women during the influenza season they attended a 20-week ultrasound was collected from the Vaccination Register. The Register of Primary Health Care Contacts and the Hospital Discharge Register include all ICD-10 diagnoses associated with primary care visits, outpatient hospital visits and hospital admissions from the public healthcare system. From those registers, data on ICD-10 diagnoses of influenza and influenza-like illness (ILI) was collected for pregnant women and their infants. The ICD-10 codes included were J09 (influenza due to identified zoonotic or pandemic influenza virus), J10 (influenza due to identified seasonal influenza virus) and J11 (influenza, virus not identified). Data on laboratory-confirmed influenza was collected from the Register of Communicable Diseases. Data was collected on filled antibiotic and oseltamivir prescriptions within four weeks of the influenza/ILI diagnosis for the infants from the Prescription Medicines Register. From the Causes of Death Register, data on influenza-associated deaths within four weeks from influenza/ILI diagnoses was collected.

Data was also collected from Landspitali University Hospital on hospitalisations, duration of hospitalisations, intensive care unit admissions

(including neonatal intensive care unit admissions) and duration of admissions, and treatment with oseltamivir during hospitalisation. Data on ICD-10 codes associated with hospitalisations was also collected to assess influenza-associated complications. Figure 6 shows how the databases were assembled.

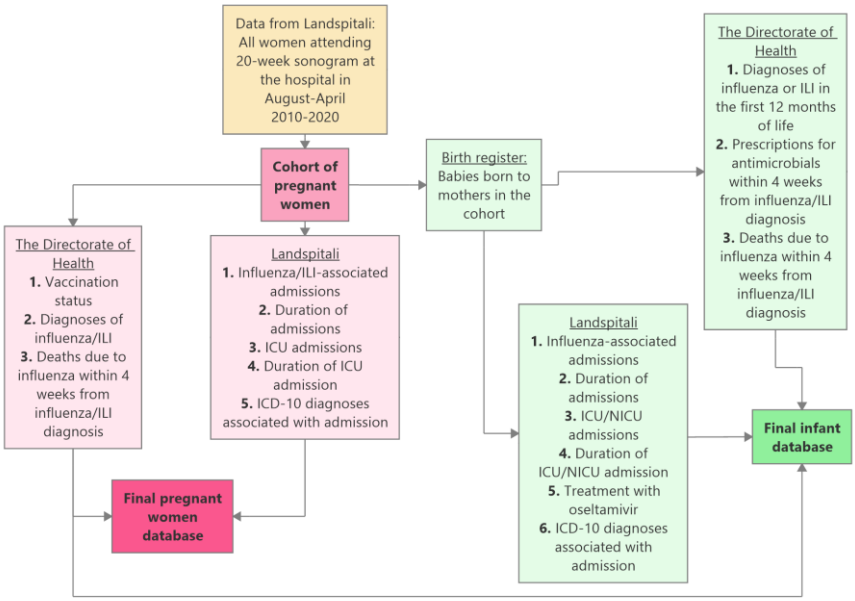

Figure 6. *Flowchart for data acquisition and database assembly for the FluRisk study.* The flowchart shows the data collection and database assembly steps for the FluRisk study.
ILI: Influenza-like illness. ICU: Intensive care unit. NICU: Neonatal intensive care unit.

3.4.2 Definitions

The influenza season was defined as 1st August to 31st May the following year. The date of the 20-week ultrasound was used to approximate the gestational age to estimate in which trimester the influenza vaccine was administered. Women were defined as vaccinated if they were vaccinated during pregnancy in the influenza season they attended a 20-week ultrasound. If a woman was vaccinated >140 days before the 20-week ultrasound, she was considered to have been vaccinated before conception but was defined as vaccinated for the influenza season. A woman was not

defined as vaccinated if she was vaccinated after giving birth. For women that did not have a registered date of delivery, they were defined as vaccinated if the vaccination was administered before an estimated 42 weeks of gestation.

Infants were considered to be born to vaccinated mothers if the mother was vaccinated >14 days before delivery. If the mother was vaccinated ≤14 days before delivery, the infants were defined as being born to unvaccinated mothers.

Repeated registrations of influenza diagnoses within 60 days were considered the same infection. When more than one influenza infection/ILI was diagnosed in the same individual, only the first infection of the season was included.

Data on influenza/ILI diagnoses and influenza-associated hospital admissions of pregnant women was included for the influenza season the woman attended a 20-week sonogram. When calculating the crude influenza incidence, the denominator for each influenza season was the number of women attending a 20-week ultrasound in the respective season. When estimating the incidence rate, days at risk were counted from 122 days prior to the date of the 20-week ultrasound or from the beginning of the influenza season (for women attending an ultrasound in August-November) to the end of the influenza season. For women diagnosed with influenza/ILI, days at risk were counted to the date of the influenza/ILI diagnosis but the days after were not counted as days at risk.

Data on influenza/ILI diagnoses and influenza/ILI-associated hospitalisations in the first year of life was included for the infants. Analyses were done for infants in the season the mother attended a 20-week ultrasound (hereafter referred to as the season of maternal vaccination), infants <6 months of age and infants <12 months of age. For incidence estimation, the infants were considered at risk in the influenza seasons the first six months and first 12 months of life overlapped, as applicable. Likewise, when estimating the number of days at risk, days at risk in both influenza seasons were counted, but days outside of influenza seasons were excluded. In cases of influenza/ILI infections, days at risk for the season were counted until the diagnosis, but the remaining days of the season were not counted as days at risk. The infant was considered at risk again from the beginning of the next season up until the age of 6 or 12 months, as applicable.

3.4.3 Data analysis and statistics

R statistical software (version 4.2.2), STATA (versions 13.0 and 17.0) and Microsoft Excel were used for data and statistical analyses. The crude incidence, and the incidence rate (IR) per 1,000 person-years were calculated for each influenza season. The incidence rate ratios (IRRs) were estimated, stratified by influenza season. The χ^2 test of heterogeneity was used to estimate IRR heterogeneity between seasons. The Mantel-Haenszel method was used to estimate a combined IRR for the ten seasons if the IRRs were not heterogenous. Vaccine effectiveness was estimated using the formula: VE = (1-IRR) x 100. For comparisons of categorical variables, Fisher's exact test and the χ^2 test were used. Mann-Whitney *U* test was used for comparison of numerical variables. A *p*-value of <0.05 and confidence intervals not overlapping 1 were considered statistically significant.

3.5 Ethics

The studies were approved by the National Bioethics Committee (Study I - ref. no. VSN-16-152; Study II – ref. no. VSN-19-017; Study III - ref. no. 19-087-V3, Study IV – ref. no. VSN-21-094) and Landspitali University Hospital Scientific Research Committee (Study I – ref. no. LSH 77-16; Study II – ref. no. 16-2019; Study III – ref. no. 16-2019; Study IV – ref. no. 16-2021). Later adaptations of the studies were also approved by the ethical committees. Study III was registered at the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT no. 2018-004386-14) and approved by the Icelandic Medicines Agency (ref. no. 2018-004386-14). The Directorate of Health, Iceland, also approved the use of data from its registers for study II (ref. no. 2203246/5.6.1.) and study IV (ref. no. 2103174/5.6.1.). Study participation was contingent upon a signed informed consent form from participants and/or their parents/guardians, as applicable by the age of the participants. Studies were conducted in accordance with the Declaration of Helsinki.

4 Results

4.1 Study I – RICE: Burden of rotavirus disease in young children in Iceland

4.1.1 Acute gastroenteritis leading to emergency department visits

A total of 1081 children <6 years of age visited the paediatric emergency department due to AGE in the two study years, 468 in 2017 and 613 in 2018. Signed informed consent was obtained for 348 children (32.2%). Twenty-three participants were excluded from the analysis; six did not meet the definition of AGE (vomiting or diarrhoea reported for >14 days before the ED visit), no samples were collected from 14 participants, and data on the duration of illness was not available for three participants. Therefore, 325 children <6 years of age were included in the study; 126 that presented to the ED in 2017 and 199 in 2018 (26.9% and 32.5% of children attending the ED due to AGE, respectively). Sixty per cent of participants (194/325) were male and 40% (131/325) were female (Table 2). The median age was 16 months (range 1-71 months). Most patients presenting to the ED with AGE were 24 months old or younger (75.7%). Seventy-two per cent of study participants attended daycare. Twenty-four participants (7.4%) had taken antibiotics in the two weeks preceding the ED visit.

The median Vesikari-score of AGE episodes leading to ED visits was 12 (range 4-18), with 65% of episodes defined as severe AGE (Vesikari score $\geq$ 11). The median number of days with symptoms before visiting the ED was four (range 1-14), and the median duration of illness was six days (range 1-28 days). Sixty per cent of participants received some treatment in the ED; 50% oral fluids, 31% antiemetics and 25% intravenous fluids (Table 2). Nine per cent of patients were admitted due to AGE, but 25.8% of non-admitted children had at least one subsequent ED visit in the next three days (76/295, data missing on subsequent visits for one participant). The majority of those had one subsequent visit (62/76 patients with subsequent visits, 81.6%) but two subsequent visits were recorded for nine children (11.8%) and three for five children (6.6%). The median number of days parents/guardians missed from work was four (range 0-21 days), and children missed a median of five days from day-care (range 0-21 days). Secondary infections occurred in 57% of cases (169/298, data missing for 27 households). The median number of secondary infections per household was one (range 0-7) (Table 2).

Table 2. *Characteristics of patients with acute gastroenteritis attending the Emergency Department at the Children's Hospital Iceland in 2017 and 2018.*
The table shows the characteristics of all acute gastroenteritis episodes and a comparison between rotavirus and non-rotavirus acute gastroenteritis characteristics.

	All AGE episodes N = 325	**RVAGE N = 142**	**Non-rotavirus AGE N = 183**	***p*-value**
Sex, male	194 (59.7)	83 (58.5)	111 (60.7)	0.773
Age, months	16 (1-71)	18 (1-69)	14 (1-71)	0.002
≤24 months of age, n (%)	246 (75.7)	100 (70.4)	146 (79.8)	0.069
Attending daycare*				0.284
Yes	230 (72.3)	106 (75.7)	124 (69.7)	
No	88 (27.7)	34 (24.3)	54 (30.3)	
Vesikari score category†				<0.001
Severe (≥11)	210 (64.8)	114 (80.3)	96 (52.7)	
Moderate (7-10)	99 (30.6)	27 (19.0)	72 (39.6)	
Mild (<7)	15 (4.6)	1 (0.7)	14 (7.7)	
Vesikari score, median (range)	12 (4-18)	13 (4-18)	11 (4-18)	<0.001
Days with symptoms prior to ED visit	4 (1-14)	3 (1-12)	4 (1-14)	0.005
Duration of illness, days	6 (1-28)	6 (1-18)	8 (1-28)	<0.001
Days missed from daycare*	5 (0-21)	5 (0-13)	5 (0-21)	0.676
Parental absence from work/school, days	4 (0-21)	4 (0-14)	4 (0-21)	0.476
Cases leading to secondary infections‡	169 (56.7)	78 (59.5)	91 (54.5)	0.450
Number of secondary cases within a family‡	1 (0-7)	1 (0-5)	1 (0-7)	0.835
Treatment in ED				
Any AGE treatment	196 (60.3)	119 (83.8)	77 (42.1)	<0.001
p.o. fluids	163 (50.1)	98 (69.0)	65 (35.5)	<0.001
i.v. fluids	82 (25.2)	56 (39.4)	26 (14.2)	<0.001
antiemetics	102 (31.4)	67 (47.2)	35 (19.1)	<0.001
Admissions	29 (8.9)	20 (14.1)	9 (4.9)	0.007

Numbers are presented as n (%) or median (range).
*: Data on daycare attendance was missing for seven participants, two in the RVAGE group and five in the non-rotavirus AGE group. Those not attending daycare were excluded from the analysis of days missed from daycare.
†: Data missing on Vesikari score for one child (in the non-rotavirus AGE group).
‡: Data missing on secondary infections for 27 cases (11 in the RVAGE group and 16 in the non-rotavirus AGE group).
AGE: Acute gastroenteritis. RVAGE: Rotavirus acute gastroenteritis. ED: Emergency Department. p.o.: *Per os.* i.v.: Intravenous

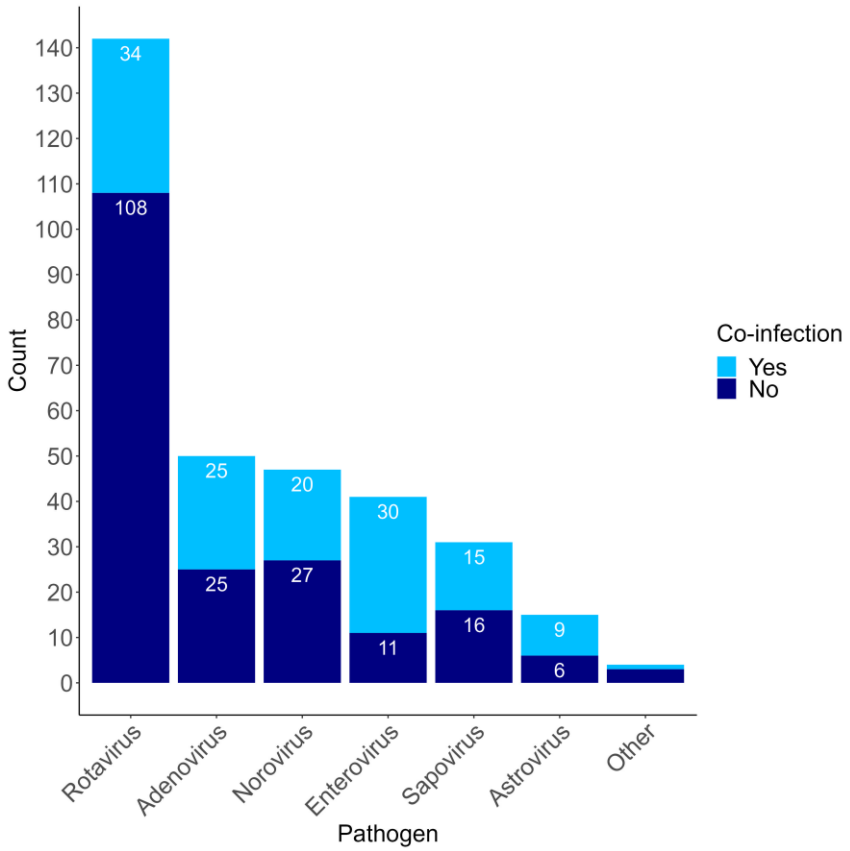

Figure 7. *Pathogens identified in acute gastroenteritis episodes among children <6 years of age, leading to emergency department visits in 2017 and 2018.*
Pathogens detected with qPCR on stool samples or rectal swabs or from stool cultures from children with acute gastroenteritis. Other: *Salmonella spp*. and *Campylobacter jejuni*.

Eighty per cent of samples yielded an identifiable pathogen (259/325). In most samples, one pathogen was identified (196/325), but two pathogens were identified in 16.9% of samples (55/325), and three in 2.5% of samples (8/325). Rotavirus was the most frequently detected virus in AGE episodes, isolated from 142 participants (54.8% of samples with a detectable pathogen), and most cases were not co-infections. Adenovirus and norovirus were the second and third most commonly detected viruses, respectively (Figure 7). Half of the adenovirus infections occurred in co-infections with other pathogens, most frequently norovirus (eight samples) and rotavirus (six samples). Enterovirus was more commonly identified in co-infections than as

the only identified pathogen. Bacterial gastroenteritis was rare, with only four cases in the two years, three with *Salmonella spp.* and one with *Campylobacter jejuni* (Figure 7). Norovirus was also identified in one of the samples with *Salmonella spp.*

More children with co-infections attended day-care than children with single pathogen infections (p = 0.005) (Table 3). Co-infections were not more serious than infections with one identified pathogen (median Vesikari score was 12 for both groups, p = 0.842). Patients with a single-pathogen infection were more frequently admitted than patients with co-infections (11.2% vs 9.52%, p = 0.007) (Table 3).

Acute gastroenteritis presentations to the ED were more common in 2018 than 2017 (Figures 8 and 9). A distinct seasonality was observed for some of the identified viruses. Rotavirus was most common in January to May, norovirus in October and November, and astrovirus was only identified in the first four months of the year (Figure 8). Infections with adenovirus were most common in the autumn and winter (September-February). Seasonality patterns were not observed for enterovirus or sapovirus (Figure 8).

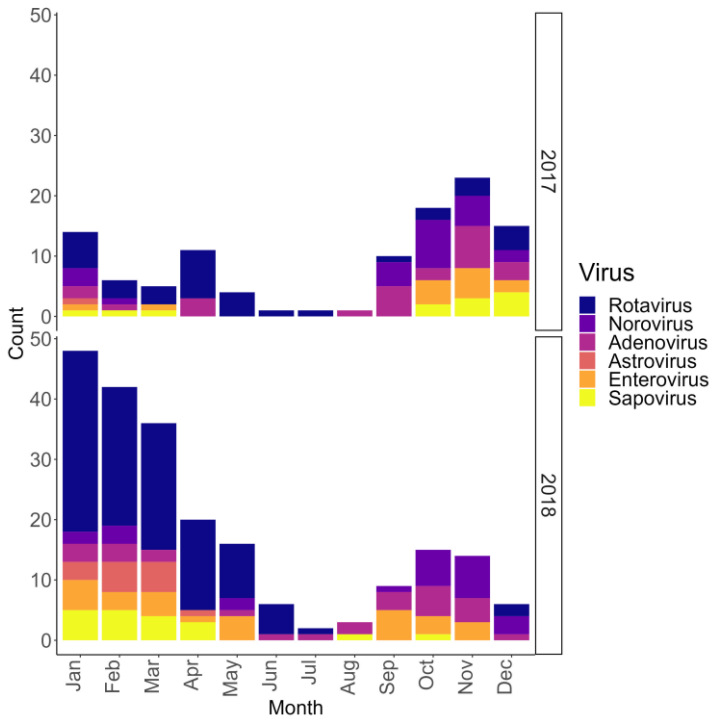

Figure 8. *Seasonality of viruses causing acute gastroenteritis.*
Number of specimens with each detected virus by month and the two study years.

Table 3. *Comparison of co-infection and single-pathogen acute gastroenteritis episodes.*

The table shows the comparison between episodes in which a single pathogen was identified and co-infections with more than one pathogen. Samples with no identified pathogen were excluded.

	Co-infections N = 63	**Single pathogen infections N = 196**	***p*-value**
Sex, male	40 (63.5)	117 (59.7)	0.698
Age, months	16 (2-58)	16 (1-69)	0.625
Attending daycare*			0.0046
Yes	56 (90.3)	139 (71.6)	
No	6 (9.7)	55 (28.4)	
Disease severity, Vesikari score†			0.266
Severe (≥11)	49 (79.0)	136 (69.4)	
Moderate (7-10)	11 (17.7)	55 (28.1)	
Mild (<7)	2 (3.2)	5 (2.6)	
Vesikari score†	12 (4-16)	12 (5-18)	0.842
Days with symptoms prior to ED visit	3 (1-14)	4 (1-14)	0.086
Duration of illness, days	6 (2-28)	6 (1-28)	0.780
Days missed from daycare*	5 (0-15)	5 (0-21)	0.112
Parental absence from work/school, days	4 (0-15)	4 (0-21)	0.622
Cases leading to secondary infections‡	40 (70.2)	110 (59.5)	0.242
Number of secondary cases within a family‡	1 (0-5)	1 (0-7)	0.212
Treatment in ED			
Any AGE treatment	49 (77.8)	126 (64.3)	0.066
p.o. fluids	43 (68.3)	101 (51.5)	0.029
i.v. fluids	23 (36.5)	54 (27.6)	0.232
antiemetics	27 (42.9)	67 (34.2)	0.274
Admissions	6 (9.52)	22 (11.2)	0.007

Numbers are presented as n (%) or median (range).

*Data on daycare attendance was missing for three participants, one in the co-infection group and two in the single-pathogen group. Those not attending daycare were excluded from the analysis of days missed from daycare.

†Data was missing on the Vesikari score for one child (in the co-infection group). ‡Data was missing on secondary infections for 17 cases (6 in the co-infection group and 11 in the single-pathogen group).

AGE: Acute gastroenteritis. ED: Emergency Department. p.o.: *Per os.* i.v.: Intravenous

4.1.2 Rotavirus acute gastroenteritis

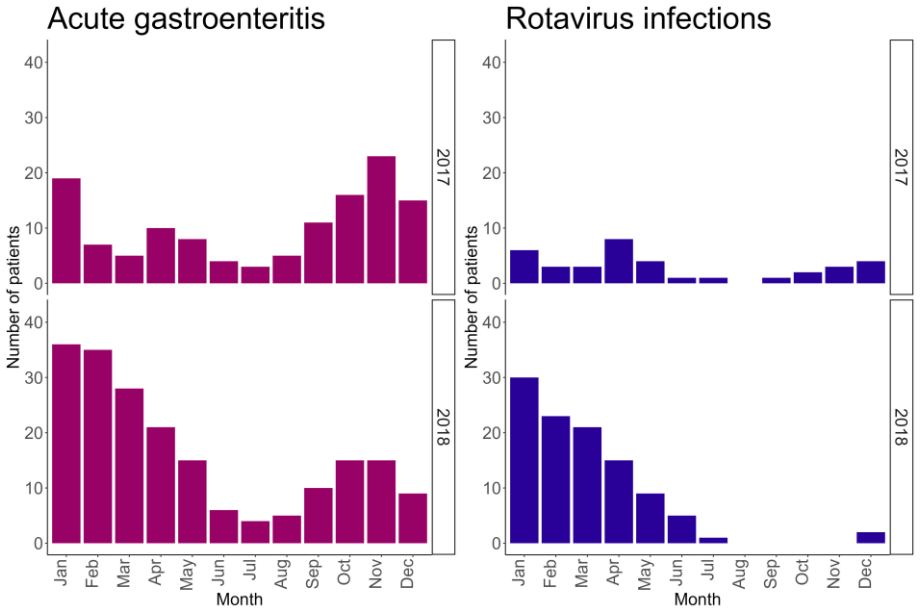

Figure 9. *Emergency department visits due to acute gastroenteritis (all-cause) and rotavirus acute gastroenteritis by month and year.*
Monthly variation in the number of patients presenting to the paediatric emergency department with acute gastroenteritis (irrespective of the pathogen) (left) and rotavirus acute gastroenteritis (right) over the two study years. Acute gastroenteritis was most prevalent in the winter months and was more prevalent in 2018 than in 2017. Rotavirus acute gastroenteritis was more prevalent, both in absolute and relative numbers, in 2018 than in 2017. Most rotavirus acute gastroenteritis cases were diagnosed in late winter and early spring.

Rotavirus was more common in 2018 than in 2017, both in absolute numbers and relative to all AGE cases (Figure 9). Rotavirus was detected in 36 (28.6%) samples in 2017 and 106 (53.3%) in 2018.

Children with RVAGE were older than children with non-rotavirus AGE, with a median age of 18 months (range 1-69 months) compared to a median of 14 months (range 1-71 months) (p = 0.002). Children with RVAGE presented to the ED after fewer days with symptoms but had a shorter duration of illness than children with non-rotavirus AGE. RVAGE cases presented to the ED after a median of three days with symptoms (range 1-12 days) compared to four days (range 1-14 days) for non-rotavirus AGE (p = 0.005). The duration of RVAGE was a median of six days (range 1-18 days)

compared to a median of eight days (range 1-28 days) for non-rotavirus AGE (p <0.001). Furthermore, more RVAGE cases were severe (80.3%) than non-rotavirus AGE cases (52.7%). Patients with RVAGE more often required treatment in the ED (84% vs 42%, p <0.001). Hospital admissions were needed in 14.1% of RVAGE cases, compared to 4.9% of non-rotavirus AGE cases (p = 0.007). In contrast, no difference was observed in the number of lost parental workdays, days missed from daycare, the proportion of cases leading to secondary household cases and the median number of secondary cases (Table 2).

4.1.3 Parental views on rotavirus vaccinations

Parents were asked about their opinion on rotavirus vaccinations. Ninety-seven per cent of parents answered the question, of which 91% were in favour of including rotavirus vaccinations in the national immunisation programme. In contrast, 6.3% of parents were against it, and 2.5% were undecided.

4.1.4 Cost of RVAGE

The number of RVAGE cases among Icelandic children under the age of six years was estimated to be 2,300 each year, based on annual attack rates from other countries. Secondary household cases occur for approximately 50% of primary cases, leading to additional 1,150 cases of RVAGE annually. If parents/guardians miss three days of work for each primary case of RVAGE, RVAGE leads to 6,900 missed workdays each year. Secondary cases were not included in the analysis of lost workdays. The cost due to lost workdays was estimated at €2.5 million annually. RVAGE ED visits and hospital admissions cost on average €369,677 per year for the two study years. Therefore, the annual cost for society due to RVAGE amounts to approximately €2.9 million.

4.2 Study II – MENICE: Meningococcal colonisation in children, adolescents, and young adults in Iceland

4.2.1 Meningococcal colonisation

A total of 1182 samples were collected in the spring of 2019: 460 from children attending DCCs, 197 from adolescents and 525 from young adults (Table 4). The mean age of the DCC children was 3.7 years (SD 1.2), 15.8 years for the adolescents (SD 0.28) and 18.9 years (SD 0.59) for the young adults. Half of the participating DCC children were female, 57% of the adolescent participants and 61% of the young adults (Table 4).

Table 4. *Participant characteristics in study II.*
The table shows participant characteristics in the study of meningococcal colonisation in Iceland. Data on sex was missing for one young adult.

	DCC children	Adolescents	Young adults
Number of samples	460	197	525
Age, mean (SD)	3.7 (1.2)	15.8 (0.28)	18.9 (0.59)
Female sex, n (%)	230 (50.0%)	112 (56.9%)	319 (60.8%)
Meningococcal carriers	0 (0.0%)	1 (0.5%)	34 (6.5%)

DCC: Daycare centre. SD: Standard deviation.

No meningococcal colonisation was detected among children attending daycare centres. Three adolescents (3/197, 1.5%) and 34 young adults (34/525, 6.5%) were identified as meningococcal carriers with culture methods. One of the three samples from adolescents was positive on qPCR (1/197, 0.51%). Both qPCR-negative samples from the adolescents had MALDI-TOF MS scores <2. All culture positive samples from the young adults were also qPCR positive. Thus, a total of 35 meningococcal carriers were identified.

Nine of the 35 carriers, all young adults, were colonised with genogroupable meningococci (25.7%). MenB was identified in six participants (6/35, 17.1%), MenY in two (2/35, 5.7%) and MenW in one (1/35, 2.9%). The other 26 carriers were colonised with non-groupable meningococci. Comparable carriage was observed between male and female participants (2.4% for females and 2.7% for males when all age groups were pooled; 6.6% for young adult females and 6.3% for young adult males, $p = 1$). Carriage was similar in two of the three colleges, 7.3% in college A (12/165) and 7.6% in college B (17/223), but lower in college C (3.6%, 5/137) ($p = 0.29$) (Table 5).

In addition to the culture-positive swabs, qPCR was done on 73 culture-negative swabs (0 from DCC children, 19 from adolescents and 54 from young adults). One culture-negative sample from a young adult was positive on qPCR (1/73, 1.4%).

Table 5. *Characteristics of meningococcal carriers and non-carriers.*

	Carriers (N = 35)	**Non-carriers (N = 1147)**	***p*-value**
Age group			<0.001
DCC children	0 (0%)	460 (40.1%)	
Adolescents	1 (2.9%)	196 (17.1%)	
Young Adults	34 (97.1%)	491 (42.8%)	
Sex – female	21 (60.0%)	642 (56.0%)	0.76
College (young adults only)	N = 34	N = 491	0.29
College A	12 (35.3%)	153 (31.2%)	
College B	17 (50.0%)	206 (41.9%)	
College C	5 (14.7%)	132 (26.9%)	

4.2.1.1 Longitudinal follow-up of meningococcal carriers

Follow-up samples were collected from carriers at three- to-six-month intervals until the first negative swab. The number of collected samples, positive samples and the identified capsular groups are shown in Figure 10. Due to restrictions because of the COVID-19 pandemic, not all samples could be collected at the 12-month follow-up. All identified carriers at the six-month follow-up were, therefore, invited back for the 15-month follow-up.

Twenty-seven individuals participated in the longitudinal part of the study. Of those, 23 (85.2%) still carried meningococci three months after collection of the first sample (Figure 10, Table 6), and after six months, 66.7% of participants were still carriers (10/15). After 15 months, 3/8 participants remained colonised. The same genogroup was identified in all successive samples from the same carrier (Table 6). One follow-up sample (ID 0305-2) was not available for qPCR but was culture positive with a MALDI-TOF MS score >2. The longest duration of carriage, which was with non-groupable meningococci, was at least 21 months (from the first positive to the last positive sample). The longest duration of carriage of genogroupable meningococci (MenB) was at least 12 months (Figure 10, Table 6).

Overall, non-groupable meningococci were most common (62/77, 80.5%), followed by MenB (12/77, 15.6%). MenY were identified in two samples (2/77, 2.6%) and MenW in one (1/77, 1.3%). Both participants carrying MenY had negative follow-up swabs after three months, and the one carrier colonised with MenW was lost to follow-up.

Table 6. *Longitudinal follow-up of meningococcal carriers.*

The longest duration of carriage, from the first to the last positive swab, was 21 months. The longest duration of carriage of capsulated meningococci was 12 months. Filled antibiotic prescriptions between follow-up swabs are denoted in the table with symbols, with different symbols for antibiotics potentially effective against *Neisseria meningitidis* and antibiotics not effective against *N. meningitidis* (see key below the table).

ID	1st swab 1	3 Months 2	6 Months 3	12 Months 4	15 Months 5	18 Months 6	21 Months 7	27 Months 8
0005	NG	NG	NG	No sample*	Neg.			
0011	NG	NG	Neg.					
0022	NG	NG	Neg.					
0025	NG	NG	Neg.					
0038	NG	NG	NG †	NG	Neg.			
0067	NG	NG	NG	NG	NG ‡	Neg.		
0074	**MenY**	Neg.						
0081	**MenB**	**MenB** †	Neg.					
0103	NG	NG	Lost to follow-up					
0107	NG	Lost to follow-up						
0129	NG	Lost to follow-up						
0141	**MenB**	Lost to follow-up						
0169	NG	NG	Lost to follow-up					
0174	NG	NG	Lost to follow-up					
0190	NG	NG	NG	No sample*	Neg.			
0191	NG	NG	Lost to follow-up					
0251	NG	Neg.						
0252	NG	NG	Lost to follow-up					
0254	**MenB**	**MenB**	**MenB**	**MenB**	Neg.			
0258	NG ‡	NG †	Neg.					
0259	NG	NG ‡	NG	No sample*	Lost to follow-up			
0267	NG	Lost to follow-up						

ID	1st swab 1	3 Months 2	6 Months 3	12 Months 4	15 Months 5	18 Months 6	21 Months 7	27 Months 8
0297	NG	NG ‡	NG	NG	Neg.			
0302	**MenY**	Neg.						
0305	NG	Culture pos.§	Lost to follow-up					
0312	NG	Neg.						
0328	**MenB**	Lost to follow-up						
0338	NG	NG ‡	NG ‡	NG	Lost to follow-up			
0347	**MenB**	**MenB**	Lost to follow-up					
0430	NG	NG	NG	No sample*	NG	Lost to follow-up		
0450	NG	Lost to follow-up						
0503	**MenW**	Lost to follow-up						
0504	**MenB**	**MenB**	Lost to follow-up					
0536	NG	Lost to follow-up						
0571	NG	NG	NG	No sample*	NG	NG	NG	Neg.

NG = non-groupable. MenB = *Neisseria meningitidis* genogroup B. MenY = *Neisseria meningitidis* genogroup Y. MenW = *Neisseria meningitidis* genogroup W.
*: The collection of swabs at the 12-month follow-up was unfinished due to restrictions because of the COVID-19 pandemic.
†: Antibiotic use, effective against *Neisseria meningitidis* (phenoxymethylpenicillin, amoxicillin, amoxicillin-clavulanic acid), following the sampling time point.
‡: Antibiotic use, not effective against *Neisseria meningitidis* (clindamycin, dicloxacillin, cephalexin and mecillinam), following the sampling time point.
§: Culture-positive but sample not available for qPCR.

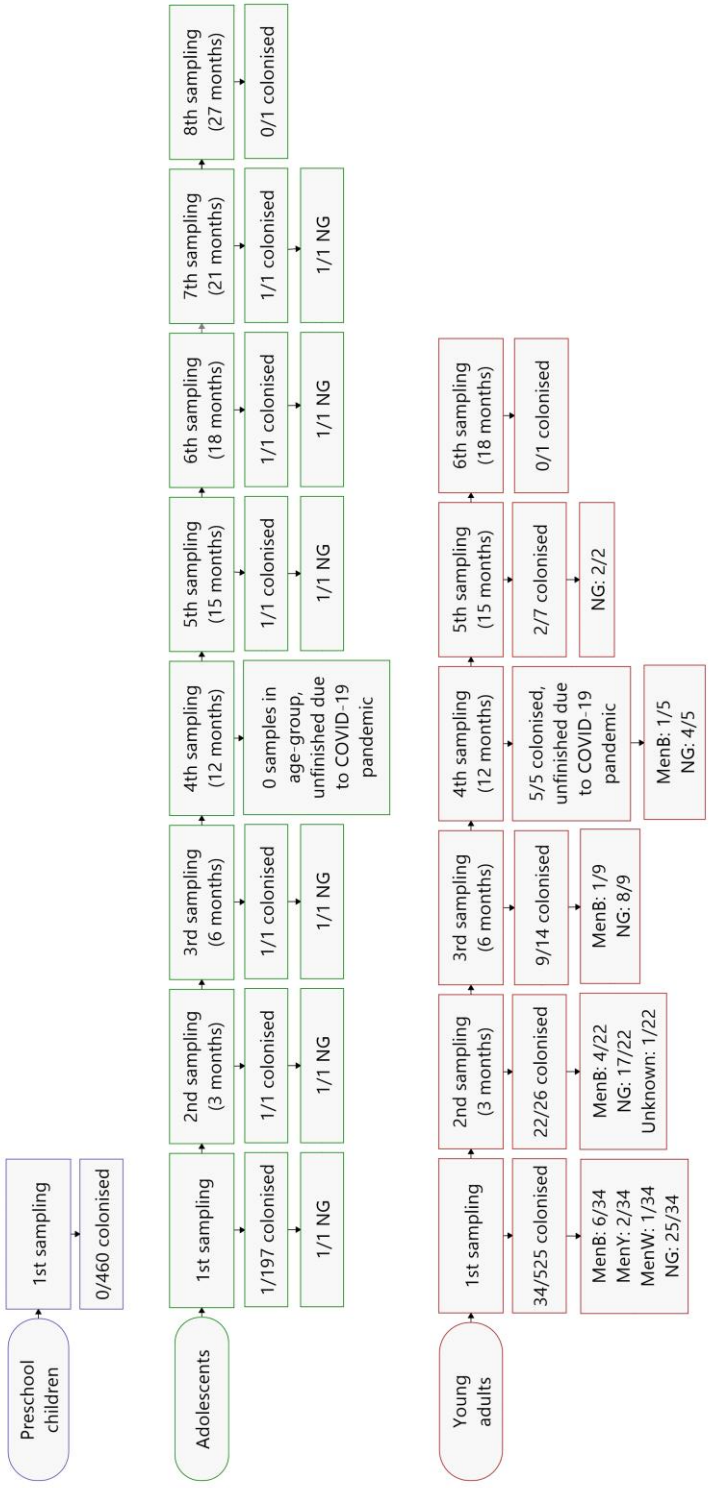

Figure 10. *Collected samples and samples positive for N. meningitidis at each sampling time point.*
The figure shows the number of samples collected, samples positive for *N. meningitidis* and the identified capsular groups at each sampling time point by age group.

Neither the absolute abundances (median $7.54*10^{-6}$ ng/µl vs $9.14*10^{-6}$ ng/µl, $p = 0.50$) nor relative abundances (0.000087 vs 0.00107, $p = 0.45$) of *N. meningitidis* were higher in the first samples from prolonged carriers compared to non-prolonged carriers. However, the absolute abundances of *N. meningitidis* (median $5.58*10^{-5}$ ng/µl vs $7.58*10^{-6}$ ng/µl, $p = 0.004$) and the relative abundances (median 0.0005 vs 0.00006, $p = 0.001$) were higher in follow-up samples compared to first samples.

4.2.2 Meningococcal vaccinations

Data was not collected on meningococcal vaccine uptake for the DCC children, but the MenC vaccine uptake in the NIP is around 94%.[185] Of the adolescents and young adults, 582 (80.6%) participants had been vaccinated against meningococci, of which 580 were vaccinated with a monovalent MenC vaccine (Table 7). Six participants had been vaccinated with a quadrivalent MenACWY vaccine, but vaccinations against MenB were not registered for any participant. No meningococcal vaccinations were registered for 140 (19.4%) participants.

Eighty-nine per cent (175/197) of the adolescents had been vaccinated against meningococci, 174 with a monovalent MenC vaccine and one with an unspecified monovalent meningococcal vaccine. Most adolescents (150/197, 76.1%) had received two doses of the monovalent MenC vaccine, according to the NIP. Twenty-three adolescents (11.7%) were vaccinated with one dose of a monovalent MenC vaccine, and one received three doses. Two adolescents were additionally vaccinated with one dose of a quadrivalent MenACWY vaccine (Table 7).

Of the young adults, 407 (77.5%) had been vaccinated against meningococci. One had been vaccinated with one dose of the quadrivalent MenACWY vaccine only; the other 406 (77.3%) had been vaccinated with a monovalent MenC vaccine. Four hundred (76.2%) had received one dose of the monovalent vaccine, according to the catch-up campaign, and six (1.14%) had received two doses. Three were additionally vaccinated with one dose of a MenACWY vaccine.

Overall, thirty meningococcal carriers had been vaccinated against meningococci (85.7% of carriers). The other five carriers (14.3%) had no registered meningococcal vaccinations. Of the non-carriers, 552 (80.3%) had been vaccinated, and 135 (19.7%) had not. Meningococcal vaccinations were not associated with decreased risk of colonisation with any meningococci (OR 1.47, 95% CI 0.55-4.93, $p = 0.52$) (Table 7).

Table 7. *Vaccinations against meningococci among the adolescent and young adult participants.*

	Total (N = 722)	**Carriers (N = 35)**	**Non-carriers (N = 687)**	***p*-value**
Vaccination against meningococci	582/722 (80.6%)	30/35 (85.7%)	552/687 (80.3%)	0.52
MenC vaccine	580/722 (80.3%)	30/35 (85.7%)	550/687 (80.1%)	
MenACWY vaccine	6/722 (0.83%)	0	6/687 (0.87%)	
Unspecified monovalent vaccine	1/722 (0.14%)	0	1/687 (0.15%)	
No registered vaccinations	140/722 (19.4%)	5/35 (14.3%)	135/687 (19.7%)	
Vaccinated adolescents	175/197 (88.8%)	1/1 (100%)	174/196 (88.8%)	1.0
Monovalent MenC vaccine	174/197 (88.3%)	1/1 (100%)	173/196 (88.3%)	
1 dose	23/197 (11.7%)	0	23/196 (11.7%)	
2 doses	150/197 (76.1%)	1/1 (100%)	149/196 (76.0%)	
3 doses	1/197 (0.5%)	0	1/196 (0.5%)	
Unspecified monovalent vaccine	1/197 (0.5%)	0	1/196 (0.5%)	
Quadrivalent MenACWY vaccine	2/197 (1.0%)	0	2/196 (1.02%)	
Vaccinated young adults	407/525 (77.5%)	29/34 (85.3%)	378/491 (77.0%)	0.39
Monovalent MenC vaccine	406/525 (77.3%)	29/34 (85.3%)	377/491 (76.8%)	
1 dose	400/525 (76.2%)	29/34 (85.3%)	371/491 (75.6%)	
2 doses	6/525 (1.1%)	0/34	6/491 (1.22%)	
Quadrivalent MenACWY vaccine	4/525 (0.76%)	0/34	4/491 (0.81%)	

4.2.3 Antibiotic use

Forty-four children attending DCCs (9.6%) had been treated with antibiotics in the 30 days prior to sample collection, according to a questionnaire filled out by parents/guardians. Seven children (1.5%) were treated with antibiotics on the sampling day.

Filled antibiotic prescriptions in the 30 days preceding the swab were registered for 36 adolescents and young adults (36/722, 5%): 12/197 adolescents (6.1%) and 24/525 young adults (4.6%). Thirty participants (30/722, 4.2%) had filled prescriptions for antibiotics potentially effective against meningococci (phenoxymethylpenicillin, amoxicillin, amoxicillin-clavulanic acid, doxycycline, lymecycline, azithromycin) but the other six (6/722, 0.8%) had filled prescriptions for antibiotics not effective against *N. meningitidis* (clindamycin, cloxacillin, cephalexin). One meningococcal carrier had filled an antibiotic prescription for cloxacillin in the 30 days prior to the swab. Of the non-carriers, 30 (4.4%) had filled prescriptions for antibiotics

that could affect carriage, whereas five (0.7%) had filled prescriptions for antibiotics not effective against meningococci.

Eight of the 23 persistent carriers filled prescriptions for antibiotics between the collection of follow-up samples (Table 6). Four were prescribed antibiotics effective against meningococci (phenoxymethylpenicillin, amoxicillin, amoxicillin-clavulanic acid), and three of the four had negative samples after the antibiotic treatment (Table 6).

4.2.4 Tonsillectomies

Ninety-two (92/722, 12.7%) adolescent and young adult participants had undergone a tonsillectomy. Four of them, all non-carriers, had the procedure in 2019. They were excluded from the analysis since it could not be ascertained whether the procedure was done before or after the sample collection. Of those that had undergone a tonsillectomy, 11.4% were meningococcal carriers, compared to 4.0% of those that had not had a tonsillectomy (Table 8).

Table 8. *Comparison between participants who had undergone tonsillectomy and those that had not*.
The table shows a comparison of the adolescent and young adult participants that had undergone a tonsillectomy before 2019 and those that had not.

	Tonsillectomy, N = 88	**No tonsillectomy, N = 630**	***p*-value**
Sex, male*	38 (43.2%)	251 (39.8%)	0.638
Age, mean (SD)	18.5 (1.4)	18.0 (1.5)	0.0005
Vaccinated against meningococci	71 (80.7%)	508 (80.6%)	1.00
Antibiotic use in the past 30 days	3 (3.4%)	33 (5.2%)	0.607
Meningococcal carrier	10 (11.4%)	25 (4.0%)	0.006

*Data on sex was missing for one participant that had not undergone a tonsillectomy.

Of meningococcal carriers, 10/35 (28.6%) had undergone a tonsillectomy compared to 78/687 (11.4%) of non-carriers (p = 0.006). The mean number of years since the tonsillectomies were 9.9 years for meningococcal carriers (SD 5.1) and 9.3 years for non-carriers (SD 4.8) (p = 0.68). Tonsillectomies were associated with a three-fold increase in the risk of meningococcal carriage (OR 3.10, 95% CI 1.44-6.70, p = 0.004), and age was also

associated with increased carriage risk (OR 1.81, 95% CI 1.28-2.56, p = 0.0008). After adjusting for age, sex, antibiotic use in the past 30 days and meningococcal vaccinations, tonsillectomies remained an independent risk factor for meningococcal colonisation (aOR 2.49, 95% CI 1.13-5.48, p = 0.024) (Table 9).

Swabs from carriers that had undergone tonsillectomies contained similar absolute and relative abundances of *N. meningitidis* as swabs from carriers that had not had tonsillectomies (mean absolute abundance $3.54*10^{-5}$ ng/µl vs $3.22*10^{-5}$ ng/µl, p = 0.54 and mean relative abundance $1.9*10^{-4}$ vs $4.9*10^{-4}$, p = 0.84).

Table 9. *Logistic regression analyses of risk factors for meningococcal carriage.*
The table shows the univariable and multivariable logistic regression analyses for risk factors for meningococcal carriage. The multivariable analysis included the variables sex, age, vaccination against meningococci (yes/no), antibiotic use in the past 30 days (yes/no) and tonsillectomy (yes/no).

	Unadjusted OR (95% CI)	***p*-value**	**Adjusted OR (95% CI)**	***p*-value**
Sex, male	0.99 (0.49-1.97)	0.970	0.90 (0.44-1.83)	0.769
Age	**1.81 (1.28-2.56)**	**0.0008**	**1.76 (1.25-2.48)**	**0.001**
Unvaccinated against meningococci	0.69 (0.26-1.81)	0.448	0.57 (0.21-1.52)	0.262
Antibiotic use in the past 30 days	0.54 (0.07-4.09)	0.555	0.66 (0.09-5.10)	0.694
Tonsillectomy	**3.10 (1.44-6.70)**	**0.004**	**2.49 (1.13-5.48)**	**0.024**

4.2.5 Whole genome sequencing

Whole genome sequencing was done on fourteen strains: ten from four prolonged carriers and four from non-prolonged carriers. One genogroupable strain (MenW according to qPCR) was non-viable and could, therefore, not be sequenced. Eight of the 14 strains (57.1%) were determined to be of genogroup B, including 7/10 strains from the prolonged carriers and 1/4 from the non-prolonged carriers (Table 10). Four strains were non-groupable with a B backbone, three of those had a phase variable off in the *csb* gene, and in the fourth, *csb* was disrupted by an insertion element (Table 10). Strains from 3/4 prolonged carriers belonged to clonal complex (CC) 213 and sequence type (ST) 213, as well as one strain from a non-prolonged carrier. The CC-213 strains were either genogroup B or non-groupable with a B backbone.

The fourth prolonged carrier carried strains belonging to CC-41/44, ST-409. Both strains with genogroup Y belonged to CC-23 but had distinct STs, ST-23 and ST-1655, respectively.

Table 10. *Whole genome sequencing of capsulated meningococci.*
Whole genome sequencing results with the genogroup, capsule locus mutations, sequence types (STs) and clonal complexes (CCs) from capsulated isolates.

SAMPLE ID	GENOGROUP	MUTATION IN CAPSULE LOCUS	ST	CC
PROLONGED CARRIAGE				
0081-1	NG – B backbone	Phase variable off in *csb*	213	213
0081-2	NG – B backbone	Phase variable off in *csb*	213	213
0254-1	B		213	213
0254-2	B		213	213
0254-3	B		213	213
0254-4	NG – B backbone	Phase variable off in *csb*	213	213
0347-1	B		213	213
0347-2	B		213	213
0504-1	B		409	41/44
0504-2	B		409	41/44
NON-PROLONGED CARRIAGE				
0074-1	Y		23	23
0141-1	NG – B backbone	*csb* disrupted by an insertion element	11527	32
0302-1	Y		1655	23
0328-1	B		213	213

Strains from the same carrier had 0-3 allelic differences, indicating a close relatedness (Figure 11). No allelic differences were found between three strains with genogroup B from carrier 0254, cultured at baseline, after three months and after six months. One allelic difference was detected in the fourth strain from carrier 0254, compared to the other three, and the fourth strain was non-groupable with a B backbone (Table 10, Figure 11). Only 3-5 allelic differences separated strains from two carriers (0081 and 0254), who were students from different colleges. No information was available on the two participants' epidemiological links. Strains belonging to CC-213 from different carriers were separated by 3-171 allelic differences, whereas allelic differences between strains of different CCs were 1260-1317.

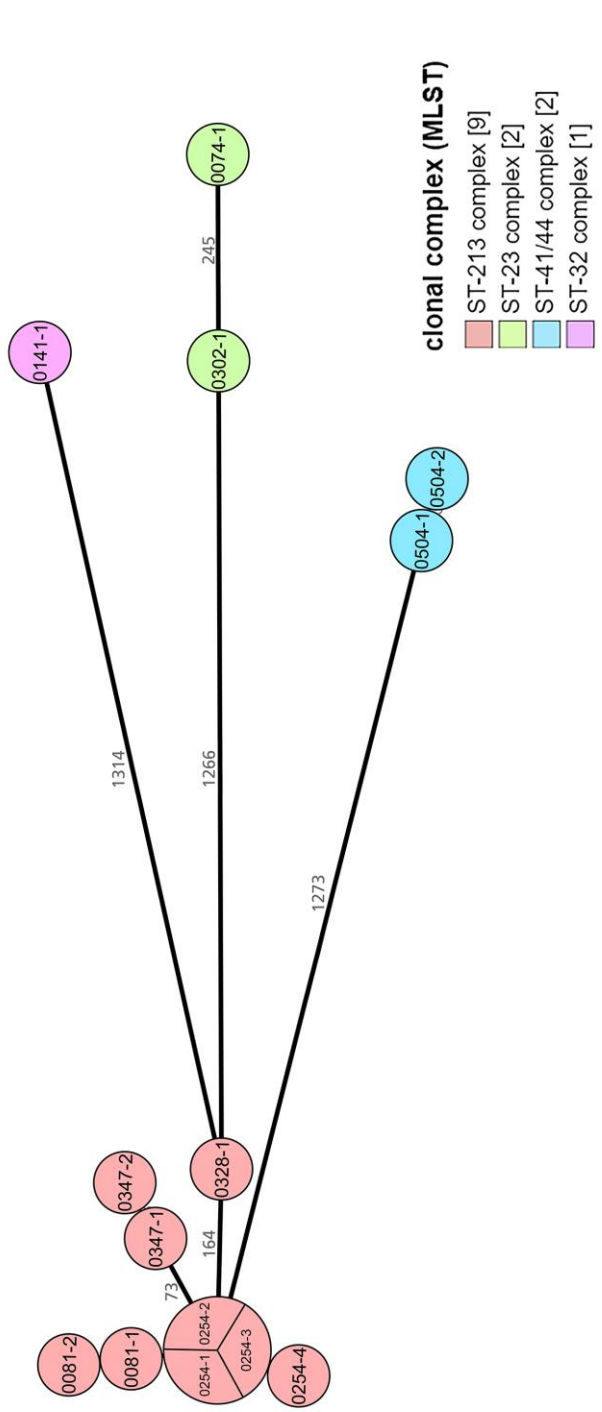

Figure 11. *Minimum spanning tree of capsulated meningococcal isolates.*
Minimum spanning tree showing the relatedness between capsulated *N. meningitidis* strains in the study, coloured by clonal complexes (CCs). The points are labelled with the sample IDs (participant ID and swab number). The number of allelic differences are shown with numbers between isolates, if larger than five. Repeated samples from the same carrier were closely related to each other. Six of the isolates with CC ST-213 were of genogroup B (0254-1, 0254-2, 0254-3, 0347-1, 0347-2) but three (0254-4, 0081-1, 0081-2) were identified as non-groupable with a B backbone. The isolate with CC ST-32 was also non-groupable with a B backbone and both isolates with CC ST-41/44 were genogroup B. Both isolates with CC ST-23 had genogroup Y.

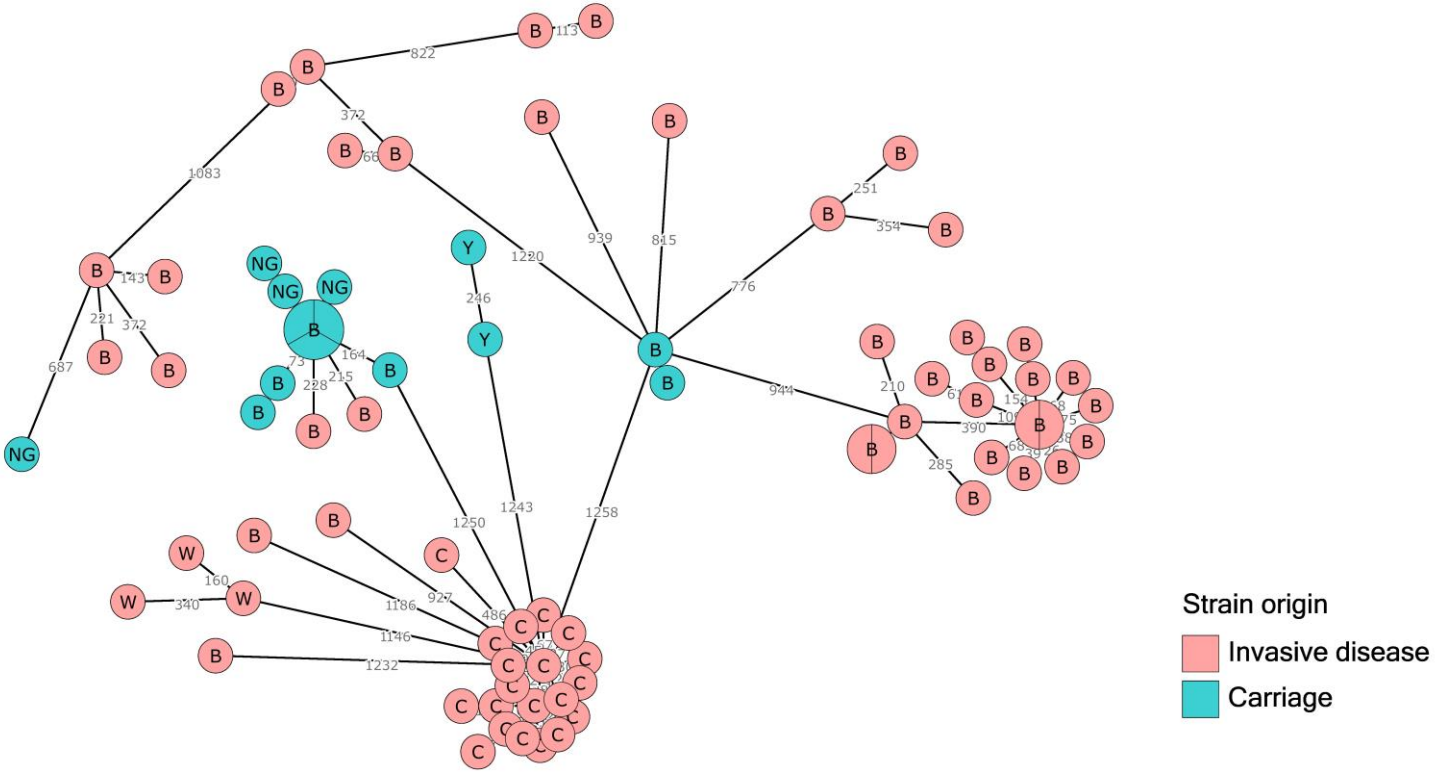

Figure 12. *The relationship between meningococcal carriage isolates from the MENICE study and Icelandic invasive meningococcal isolates registered in PubMLST.*

The carriage strains from the MENICE study are shown in blue, and strains from invasive meningococcal disease cases in Iceland, registered in PubMLST, are shown in coral. The capsular groups are shown with their corresponding letter in the circles. The numbers on the lines represent the number of allelic differences between the strains. Larger points represent more than one identical strain; the number of strains is indicated with a pie-split of the point.

Most carriage strains in our study were distantly related to invasive strains from Iceland, submitted to PubMLST (Figure 12). The closest relatedness between carriage and invasive strains were between MenB strains that had 215 and 228 allelic differences, respectively (Figure 12).

The MenDeVAR index was used to assess the reactivity of the two licenced protein-based meningococcal B vaccines against the carriage strains determined to be capsulated by qPCR. All strains with CC-213 had an exact match to a Trumenba® vaccine antigen (fHbp peptide 45), whereas Bexsero® had no reactivity to 7/9 CC-213 strains from 3/4 CC-213 carriers (Table 11). The data on Bexsero® reactivity was insufficient for the other two CC-213 strains. Trumenba® was cross-reactive to the MenB CC-41/44 strains isolated from one carrier in our study (fHbp peptide 19), but data on Bexsero® reactivity was insufficient. Trumenba® was also cross-reactive to the two MenY CC-23 strains isolated from two carriers (fHbp peptide 25) (Table 11). The Bexsero Antigen Sequence Typing (BAST) was incomplete for four strains (0081-1, 0141-1, 0504-1 and 0504-2) with 4 out of the 5 loci in the scheme designated.

Table 11. *Reactivity of the two licensed protein-based meningococcal B vaccines against antigens on the Icelandic carriage strains, according to the MenDeVAR index on PubMLST.*

Strain ID	Genogroup qPCR	Genogroup WGS	Clonal complex	Bexsero® (4CMenB) reactivity	Trumenba® (fHbp vaccine) reactivity
0074-1	Y	Y	23	Insufficient data	Cross-reactive
0081-1	B	NG	213	Insufficient data*	Exact match
0081-2	B	NG	213	Insufficient data	Exact match
0141-1	B	NG	32	Insufficient data*	Insufficient data
0254-1	B	B	213	None	Exact match
0254-2	B	B	213	None	Exact match
0254-3	B	B	213	None	Exact match
0254-4	B	NG	213	None	Exact match
0302-1	Y	Y	23	Insufficient data	Cross-reactive
0328-1	B	B	213	None	Exact match
0347-1	B	B	213	None	Exact match
0347-2	B	B	213	None	Exact match
0504-1	B	B	41/44	Insufficient data*	Cross-reactive
0504-2	B	B	41/44	Insufficient data*	Cross-reactive

*Bexsero Antigen Sequence Scheme (BAST) incomplete.

4.2.6 Concordance of MALDI-TOF MS, API NH® and qPCR in the identification of *N. meningitidis*

In total, 80 strains were identified as *N. meningitidis* with culture and MALDI-TOF MS and were subjected to qPCR. OPS qPCR was positive for *N. meningitidis* in 69/80 (86.3%) samples. Eight samples that were negative on OPS qPCR were positive on qPCR of the cultured strain, resulting in a total of 77 qPCR positive samples.

The concordance of MALDI-TOFS MS and qPCR detection of *N. meningitidis* was compared, using qPCR results of the cultured strains as a reference. Nine strains were non-viable; therefore, qPCR was done on 71 cultured strains. MALDI-TOF MS correctly identified 97% of strains as *N. meningitidis* (69/71). Two strains identified as *N. meningitidis* with MALDI-TOF MS scores of 1.84 and 1.92 were negative on qPCR (both OPS and cultured strain qPCR). The MALDI-TOF MS scores for 13% (9/69) of the strains confirmed to be *N. meningitidis* by qPCR were <2.0 (range 1.7-1.98).

Two of the 34 strains tested with API NH® had been identified as *N. meningitidis* by MALDI-TOF MS but were qPCR negative (strains 0559-1 and 0669-1, Table 12). The other 32 were confirmed by qPCR to be meningococci. API NH® confirmed the identification of *N. meningitidis* for 30/34 but identified two meningococcal strains as commensal *Neisseria spp.* (*N. polysaccharea and N. cinerea,* Table 12). API NH® identified one of the non-meningococcal strains as *N. polysaccharea* and gave an inconclusive result for the other (Table 12).

Table 12. *qPCR, MALDI-TOF MS and API NH® detection of meningococci.*
The table shows a comparison of the results from qPCR of cultured strains, MALDI-TOF MS and API NH®, for the 34 strains tested by all methods.

Strain	Definition	*metA* qPCR, cultured strain	*ctrA* qPCR, cultured strain	MALDI-TOF MS score	API NH® result
0005-1	NG	Pos.	Neg.	1.73	*N. Cinerea*
0005-3	NG	Pos.	Neg.	2.22	*N. Meningitidis*
0011-2	NG	Pos.	Neg.	2.32	*N. Meningitidis*
0022-1	NG	Pos.	Neg.	2.1	*N. Meningitidis*
0022-2	NG	Pos.	Neg.	2.47	*N. Meningitidis*
0025-1	NG	Pos.	Neg.	2.17	*N. Meningitidis*
0025-2	NG	Pos.	Neg.	2.24	*N. Meningitidis*
0038-1	NG	Pos.	Neg.	2.05	*N. Meningitidis*
0038-3	NG	Pos.	Neg.	2.19	*N. Meningitidis*
0067-3	NG	Pos.	Neg.	2.36	*N. Meningitidis*
0107-1	NG	Pos.	Neg.	2.11	*N. Polysaccharea*
0129-1	NG	Pos.	Neg.	1.89	*N. Meningitidis*
0141-1	Caps. *Nm*	Pos.	Pos.	2.11	*N. Meningitidis*
0174-2	NG	Pos.	Neg.	2.23	*N. Meningitidis*
0191-2	NG	Pos.	Neg.	1.8	*N. Meningitidis*
0251-1	NG	Pos.	Neg.	2.22	*N. Meningitidis*
0258-1	NG	Pos.	Neg.	1.95	*N. Meningitidis*
0259-3	NG	Pos.	Neg.	2.17	*N. Meningitidis*
0267-1	NG	Pos.	Neg.	2.00	*N. Meningitidis*
0297-1	NG	Pos.	Neg.	2.12	*N. Meningitidis*
0297-3	NG	Pos.	Neg.	2.19	*N. Meningitidis*
0297-4	NG	Pos.	Neg.	2.41	*N. Meningitidis*
0302-1	Caps. *Nm*	Pos.	Pos.	2.08	*N. Meningitidis*
0305-1	NG	Pos.	Neg.	1.95	*N. Meningitidis*
0312-1	NG	Pos.	Neg.	2.16	*N. Meningitidis*
0328-1	Caps. *Nm*	Pos.	Pos.	2.34	*N. Meningitidis*
0338-1	NG	Pos.	Neg.	1.94	*N. Meningitidis*
0338-4	NG	Pos.	Neg.	2.16	*N. Meningitidis*
0450-1	NG	Pos.	Neg.	2.02	*N. Meningitidis*
0504-2	Caps. *Nm*	Pos.	Pos.	2.2	*N. Meningitidis*
0536-1	NG	Pos.	Neg.	2.1	*N. Meningitidis*
0559-1	Not *Nm*	Neg.	Neg.	1.84	*N. Polysaccharea*
0571-3	NG	Pos.	Neg.	2.03	*N. Meningitidis*
0669-1	Not *Nm*	Neg.	Neg.	1.92	Inconclusive – *N. Meningitidis* / *N. Cinerea*

Caps.: Capsulated. *Nm*: *Neisseria meningitidis.* NG: Non-groupable

4.3 Study III – OFICE: Influenza vaccine responses in adolescents with obesity

4.3.1 Participants

Thirty adolescents with obesity and thirty adolescents with normal weight were recruited to participate in the study. The male:female ratio was not the same in the two groups, with 70% of the participants in the control group being male compared to 57% in the study group, although the difference was not statistically significant (Table 13). The age distribution was similar between the two groups; the median age was 14.0 years in the study group and 14.9 years in the control group. Furthermore, the median height was the same for the two groups, but as expected, there was a statistically significant difference in weight, BMI, body fat percentage and waist circumference between the two groups (Table 13). Six participants in the control group did not have normal weight, four were underweight (BMI 15.4, 15.4, 16.0 and 16.5 kg/m^2, respectively), and two were overweight (BMI 22.4 and 23.0 kg/m^2, respectively).

All 30 participants in the control group completed both visits, and 29/30 participants from the study group. One participant in the study group could not attend the second visit within the defined interval between visits and was therefore excluded from the analysis of post-vaccination measurements and seroconversion.

Table 13. *Characteristics of the study and control group in study III.*

	Study, *N* = 30	**Control, *N* = 30**	***p*-value**
Sex			0.42
Male	17 (57%)	21 (70%)	
Female	13 (43%)	9 (30%)	
Age, years	14.0 (12.3-17.8)	14.9 (12.4-17.7)	0.86
Height, cm	169 (157-188)	169 (153-188)	0.90
Weight, kg	103 (72-163)	55 (36-77)	<0.001
BMI, kg/m^2	36 (28-49)	19 (15-23)	<0.001
Waist circumference, cm	117 (96-146)	74 (62-89)	<0.001
Body fat percentage	49 (30-68)	18 (14-28)	<0.001

Numbers are presented as n (%) or median (range).
Measurements from the second visit are presented. If a measurement value was missing for the second visit, the measurement from the first visit was used.

4.3.2 Humoral immune responses

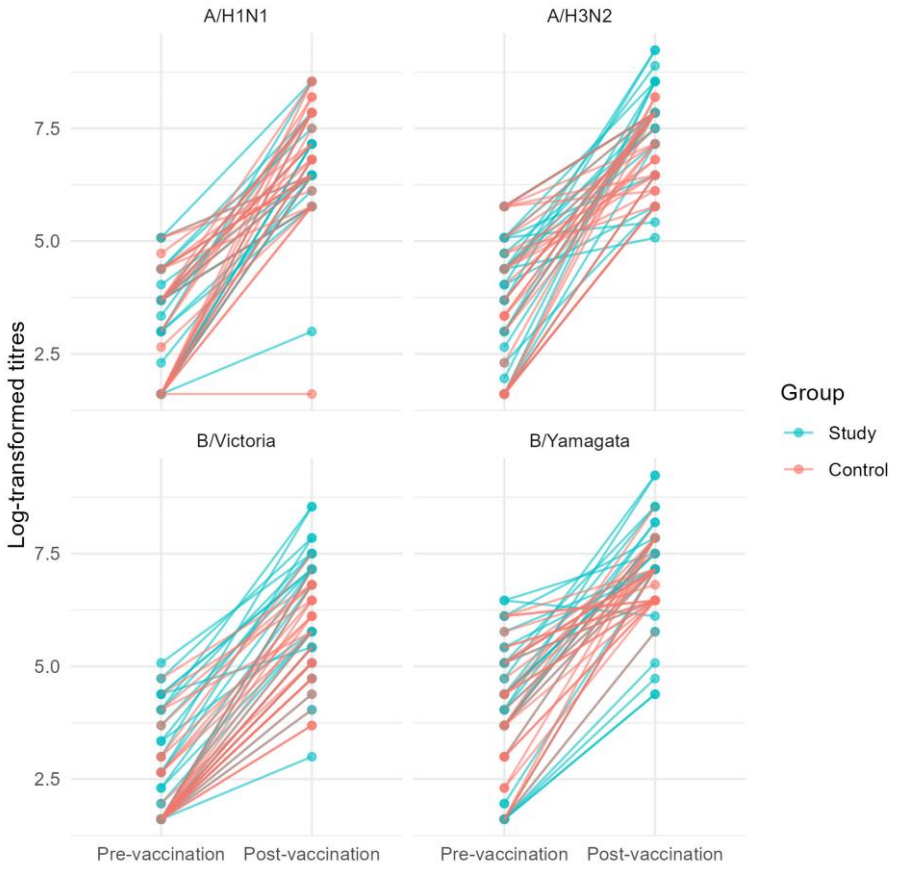

Figure 13. *Geometric mean titres pre- and post-vaccination against influenza in adolescents with obesity and adolescents with normal weight.*

Pre- and post-vaccination log-transformed geometric mean titres from haemagglutination inhibition assays by vaccine strain and group. The dots represent samples from each participant, with lines connecting the corresponding pre- and post-vaccination measurements from the same participant.

Pre-vaccination titres against all four influenza strains were similar between the two groups (Figure 13 and Table 14). The post-vaccination titres against influenza B/Victoria were higher in the study group than the control group (median 905.1 vs 273.1, p = 0.02), but after correction for multiple comparisons, the difference did not remain significant (Table 14). There was a considerable variation in the fold-increase between pre- and post-vaccination titres in both groups, but the median fold-increase was 32 for all

strains in the study group. In the control group, the fold increase was lowest for the B/Yamagata strain (a median of 16-fold increase) but highest for influenza A/H3N2 (median fold-increase 54.6) (Table 14).

A substantial proportion of participants in both groups had seroprotective titres (titres ≥40) pre-vaccination. Fifty per cent or more in both groups had seroprotective pre-vaccination titres against influenza A/H1N1, A/H3N2 and B/Yamagata. The lowest proportion of seroprotection pre-vaccination was against influenza B/Victoria, 27% in the study group and 17% in the control group (Table 14). Most participants had seroprotective titres post-vaccination, and the proportion was similar between the two groups, with 97-100% of participants having seroprotective titres for each influenza strain (Table 14). Seroconversion (≥4-fold increase in titres) occurred in 93-100% of participants in the study group for the four influenza strains and 80-100% of participants in the control group (Table 14). The seroconversion rate was lowest for the B/Yamagata strain in the control group, with 80% of participants seroconverting, compared to 93% in the study group.

Post-vaccination titres against influenza A/H3N2 and influenza B/Victoria were higher in females in the study group than females in the control group (Table 15). Furthermore, post-vaccination titres against influenza A/H3N2 were higher in males in the control group than females in the control group (Table 15). None of these differences remained significant after correcting for multiple comparisons with the Benjamini-Hochberg procedure. Other differences were not observed when comparing the responses by group and sex.

Table 14. *Comparison of the humoral immune response to influenza vaccination between adolescents with obesity and adolescents with normal weight.*

The table shows the median geometric mean titres (GMTs), fold-increase in titres after vaccination and proportion of participants reaching seroconversion (≥4 fold-increase in titres) and seroprotection (titre ≥40) for adolescents with obesity and adolescents with normal weight. One participant from the study group did not complete the study and was excluded from post-vaccination analysis of titres and seroconversion.

	Study group (N = 30 pre-vaccination, N = 29 post-vaccination)	**Control group (N = 30 pre-vaccination, N = 30 post-vaccination)**	***p*-value**
GMT pre-vaccination, median (range)			
A/H1N1	34.1 (5-160)	40.0 (5-160)	0.909
A/H3N2	68.3 (5-320)	40.0 (5-320)	0.639
B/Victoria	8.5 (5-160)	5.0 (5-113)	0.176
B/Yamagata	56.6 (5-640)	80.0 (5-453)	0.557
GMT post-vaccination, median (range)			
A/H1N1	640.0 (20-5120)	905.1 (5-5120)	0.764
A/H3N2	2560 (160-10240)	1280 (320-3620)	0.072
B/Victoria	905.1 (20-5120)	273.1 (40-1810)	**0.024***
B/Yamagata	1810 (80-10240)	1280 (320-5120)	0.115
Fold increase between pre- and post-vaccination titres, median (range)			
A/H1N1	32 (4-512)	48 (1-1024)	0.98
A/H3N2	32 (1.4-1024)	54.6 (1.4-724)	0.46
B/Victoria	32 (2.8-256)	22.6 (5.7-256)	0.28
B/Yamagata	32 (0.7-256)	16 (1.4-512)	0.32
Seroprotection pre-vaccination, n/N (%)			
A/H1N1	15/30 (50%)	16/30 (53%)	1.00
A/H3N2	20/30 (67%)	17/30 (57%)	0.596
B/Victoria	8/30 (27%)	5/30 (17%)	0.532
B/Yamagata	21/30 (70%)	21/30 (70%)	1.00
Seroprotection post-vaccination, n/N (%)			
A/H1N1	28/29 (97%)	29/30 (97%)	1.00
A/H3N2	29/29 (100%)	30/30 (100%)	
B/Victoria	28/29 (97%)	30/30 (100%)	0.492
B/Yamagata	29/29 (100%)	30/30 (100%)	
Seroconversion, n/N (%)			
A/H1N1	29/29 (100%)	28/30 (93%)	0.492
A/H3N2	27/29 (93%)	27/30 (90%)	1.0
B/Victoria	28/29 (97%)	30/30 (100%)	0.492
B/Yamagata	27/29 (93%)	24/30 (80%)	0.254

*: After adjusting for multiple comparisons with the Benjamini-Hochberg procedure, the difference did not remain significant. It was also not significant after excluding controls that did not meet the definition of being normal weight.

Table 15. *The humoral immune response to influenza vaccination by group and sex.*
The table shows a comparison of the humoral immune response in the two groups by sex and a comparison between the sexes by group. No differences remained significant after correcting for multiple testing with the Benjamini-Hochberg procedure.

	Study group	Control group	*p*-value
GMT pre-vaccination, median (range), males			
A/H1N1	20 (5-80)	40 (5-160)	0.19
A/H3N2	80 (5-320)	40 (5-320)	0.53
B/Victoria	5 (5-80)	5 (5-113)	0.58
B/Yamagata	56.6 (5-640)	80 (5-453)	0.56
GMT post-vaccination, median (range), males			
A/H1N1	773 (320-5120)	905 (320-5120)	0.59
A/H3N2	2560 (320-7241)	2560 (320-3620)	0.63
B/Victoria	320 (20-5120)	226 (40-1810)	0.41
B/Yamagata	1280 (80-5120)	1280 (320-2560)	0.44
GMT pre-vaccination, median (range), females			
A/H1N1	40 (5-160)	5 (5-40)	0.11
A/H3N2	56.6 (5-320)	80 (5-320)	0.95
B/Victoria	10 (5-160)	5 (5-56.6)	0.17
B/Yamagata	56.6 (5-640)	40 (5-453)	0.97
GMT post-vaccination, median (range), females			
A/H1N1	640 (20-5120)	640 (5-2560)	0.66
A/H3N2	2560 (160-10240)	640 (320-2560)	**0.04**
B/Victoria	1280 (160-5120)	320 (40-1280)	**0.04**
B/Yamagata	1810 (80-10240)	640 (640-5120)	0.29
Seroprotection, pre-vaccination, males			
A/H1N1	6/17 (35.3%)	12/21 (57.1%)	0.32
A/H3N2	11/17 (64.7%)	12/21 (57.1%)	0.52
B/Victoria	4/17 (23.5%)	4/21 (19.0%)	0.70
B/Yamagata	11/17 (64.7%)	15/21 (71.4%)	1.00
Seroprotection, post-vaccination, males			
A/H1N1	16/16 (100%)	21/21 (100%)	-
A/H3N2	16/16 (100%)	21/21 (100%)	-
B/Victoria	15/16 (93.8%)	21/21 (100%)	0.43
B/Yamagata	16/16 (100%)	21/21 (100%)	-
Seroprotection, pre-vaccination, females			
A/H1N1	9/13 (69.2%)	4/9 (44.4%)	0.38
A/H3N2	8/13 (61.5%)	5/9 (55.6%)	1.00
B/Victoria	4/13 (30.8%)	1/9 (11.1%)	0.36
B/Yamagata	10/13 (76.9%)	6/9 (66.7%)	0.66
Seroprotection, post-vaccination, females			
A/H1N1	12/13 (92.3%)	8/9 (88.9%)	1.00
A/H3N2	13/13 (100%)	9/9 (100%)	-
B/Victoria	13/13 (100%)	9/9 (100%)	-
B/Yamagata	13/13 (100%)	9/9 (100%)	-
Seroconversion, males			
A/H1N1	16/16 (100%)	20/21 (95.2%)	1.00
A/H3N2	16/16 (100%)	19/21 (90.5%)	0.50
B/Victoria	16/16 (100%)	21/21 (100%)	-
B/Yamagata	15/16 (93.8%)	17/21 (81.0%)	0.36
Seroconversion, females			
A/H1N1	13/13 (100%)	8/9 (88.9%)	0.41
A/H3N2	11/13 (84.6%)	8/9 (88.9%)	1.00
B/Victoria	12/13 (92.3%)	9/9 (100%)	1.00
B/Yamagata	12/13 (92.3%)	7/9 (77.8%)	0.54

	Females	Males	*p*-value
GMT pre-vaccination, median (range), study group			
A/H1N1	40 (5-160)	20 (5-80)	0.18
A/H3N2	56.6 (5-320)	80 (5-320)	1.00
B/Victoria	10 (5-160)	5 (5-80)	0.52
B/Yamagata	56.6 (5-640)	56.6 (5-640)	0.92
GMT post-vaccination, median (range), study group			
A/H1N1	640 (20-5120)	773 (320-5120)	0.65
A/H3N2	2560 (160-10240)	2560 (320-7241)	0.54
B/Victoria	1280 (160-5120)	320 (20-5120)	0.13
B/Yamagata	1810 (80-10240)	1280 (80-5120)	0.40
GMT pre-vaccination, median (range), control group			
A/H1N1	5 (5-40)	40 (5-160)	0.1
A/H3N2	80 (5-320)	40 (5-320)	0.65
B/Victoria	5 (5-56.6)	5 (5-113)	0.71
B/Yamagata	40 (5-453)	80 (5-453)	0.57
GMT post-vaccination, median (range), control group			
A/H1N1	640 (5-2560)	905 (320-5120)	0.18
A/H3N2	640 (320-2560)	2560 (320-3620)	**0.04**
B/Victoria	320 (40-1280)	226 (40-1810)	0.48
B/Yamagata	640 (640-5120)	1280 (320-2560)	0.94
Seroprotection, pre-vaccination, study group			
A/H1N1	9/13 (69.2%)	6/17 (35.3%)	0.14
A/H3N2	8/13 (61.5%)	11/17 (64.7%)	0.71
B/Victoria	4/13 (30.8%)	4/17 (23.5%)	0.70
B/Yamagata	10/13 (76.9%)	11/17 (64.7%)	0.69
Seroprotection, post-vaccination, study group			
A/H1N1	12/13 (92.3%)	16/16 (100%)	0.45
A/H3N2	13/13 (100%)	16/16 (100%)	-
B/Victoria	13/13 (100%)	15/16 (93.8%)	1.00
B/Yamagata	13/13 (100%)	16/16 (100%)	-
Seroprotection, pre-vaccination, control group			
A/H1N1	4/9 (44.4%)	12/21 (57.1%)	0.69
A/H3N2	5/9 (55.6%)	12/21 (57.1%)	1.00
B/Victoria	1/9 (11.1%)	4/21 (19.0%)	1.00
B/Yamagata	6/9 (66.7%)	15/21 (71.4%)	1.00
Seroprotection, post-vaccination, control group			
A/H1N1	8/9 (88.9%)	21/21 (100%)	0.3
A/H3N2	9/9 (100%)	21/21 (100%)	-
B/Victoria	9/9 (100%)	21/21 (100%)	-
B/Yamagata	9/9 (100%)	21/21 (100%)	-
Seroconversion, study group			
A/H1N1	13/13 (100%)	16/16 (100%)	-
A/H3N2	11/13 (84.6%)	16/16 (100%)	0.19
B/Victoria	12/13 (92.3%)	16/16 (100%)	0.45
B/Yamagata	12/13 (92.3%)	15/16 (93.8%)	1.00
Seroconversion, control group			
A/H1N1	8/9 (88.9%)	20/21 (95.2%)	0.52
A/H3N2	8/9 (88.9%)	19/21 (90.5%)	1.00
B/Victoria	9/9 (100%)	21/21 (100%)	-
B/Yamagata	7/9 (77.8%)	17/21 (81.0%)	1.00

4.3.3 Cellular immune responses

An average of 36,667 live cells were included in each experiment (Table 16). The average number of $CD4^+$ cells was 6,360 and the average number of $CD8^+$ cells was 3,773 (Table 16). Information on IFN-γ was unavailable for 30 samples due to a technical error, 22 from the study group and eight from the control group. Not all samples could be tested for all the studied cytokines with the four vaccine strains because of insufficient cell numbers. Table 18 shows the number of samples tested for each cytokine with each vaccine strain and the percentage of $CD4^+$ and $CD8^+$ cells that secreted the studied cytokines in response to stimulation with the vaccine influenza strains.

The proportion of pre-vaccination $CD4^+$ cells secreting TNF-α in response to stimulation with influenza A/H3N2 was higher in the study group than the control group, but the difference did not remain after correcting for multiple comparisons. No other differences in cytokine production were observed between the groups, neither for $CD4^+$ nor $CD8^+$ cells (Table 18, Figure 14 & Figure 15). The proportions of $CD4^+/CD8^+$ T cells were comparable between the two groups, both pre- and post-vaccination (Table 17). The proportions did change significantly after the vaccination in both groups, with a proportional increase in $CD4^+$ cells (p <0.001 for pre- vs post-vaccination comparison for both groups, data not shown).

There was a significant increase from pre- to post-vaccination proportion of $CD4^+$ cells from the study group secreting IFN-γ, TNF-α and IL-2 in response to all four vaccine strains, as well as $CD8^+$ cells secreting IFN-γ in response to stimulation with the B/Victoria strain (Table 19). For the control group, there was a significant increase in the proportion of $CD4^+$ cell secreting TNF-α in response to stimulation with influenza A/H3N2, $CD4^+$ cells secreting IL-2 when stimulated with influenza A/H1N1 and B/Victoria and $CD8^+$ cells secreting IFN-γ after B/Victoria stimulation (Table 20). None of these differences remained significant after correction for multiple testing with the Benjamini-Hochberg procedure.

Table 16. *Median and mean number of cells included in T cell stimulation experiments.*

The table shows the median and mean number of cells in each T cell stimulation experiment. Due to a technical error, information on IFN-γ was unavailable for 30 samples.

	Median cell count in the experiments (range)	**Mean cell count in the experiments (SD)**
Total cell count	168,866 (30,064-1,820,000)	213,585 (190,298)
Live cell count	25,403 (10,928-230,339)	36,667 (31,357)
Cell count $CD3^+$	3,417.5 (15-129,193)	11,602 (20,016)
Cell count $CD4^+$	1,635.5 (4-67,967)	6,360 (11,029)
Cell count $CD8^+$	1,131 (4-41,143)	3,776 (6,570)
Cell count $CD4^+$ IFN-γ	5 (0-714)	21.9 (65.1)
Cell count $CD4^+$ TNF-α	1 (0-3,925)	31.7 (207.5)
Cell count $CD4^+$ IL-2	1 (0-2,985)	40.7 (197.6)
Cell count $CD4^+$ IL-13	1 (0-35)	3.1 (5.0)
Cell count $CD8^+$ IFN-γ	3 (0-573)	14.3 (55.0)
Cell count $CD8^+$ TNF-α	0 (0-716)	9.2 (55.5)
Cell count $CD8^+$ IL-2	0 (0-144)	2.6 (13.9)
Cell count $CD8^+$ IL-13	1 (0-26)	1.8 (3.2)

Table 17. *The proportion of $CD4^+/CD8^+$ cells pre- and post-vaccination in both groups.*

The table shows the proportions of $CD4^+/CD8^+$ T cells in blood samples from participants pre-vaccination and post-vaccination. There was not a statistically significant difference in the proportions between the groups, neither pre-vaccination nor post-vaccination.

	Study	**Control**	***p*-value**
Pre-vaccination $CD4^+/CD8^+$ proportion, median (range)	1.5 (0.5-2.4)	1.2 (0.3-5.1)	0.59
Post-vaccination $CD4^+/CD8^+$ proportion, median (range)	1.8 (0.6-5.2)	2.1 (0.5-6.2)	0.19

Table 18. *T cell stimulation assay: Percentage of cells secreting cytokines in response to stimulation to the vaccine strains – study vs control group.*
The table shows the number of samples in the analysis and the median (range) percentage of $CD4^+$ and $CD8^+$ T cells secreting each measured cytokine in response to stimulation with each vaccine strain. Samples that gave a higher response to the negative control than the positive control were excluded.

	Study group, samples included	Control group, samples included	Study group: % of cells expressing cytokines	Control group: % of cells expressing cytokines	*p*-value
Pre-vaccination $CD4^+$ IFN-γ					
A/H1N1	12	11	0.019 (0.003-0.051)	0.006 (0.000-0.028)	0.085
A/H3N2	12	8	0.009 (0.000-0.022)	0.004 (0.000-0.031)	0.231
B/Victoria	11	6	0.009 (0.000-0.033)	0.005 (0.000-0.023)	0.208
B/Yamagata	10	3	0.012 (0.000-0.025)	0.003 (0.000-0.008)	0.061
Post-vaccination $CD4^+$ IFN-γ					
A/H1N1	14	20	0.048 (0.003-0.092)	0.017 (0.000-0.120)	0.059
A/H3N2	14	18	0.024 (0.013-0.082)	0.022 (0.000-0.110)	0.278
B/Victoria	14	16	0.028 (0.003-0.075)	0.035 (0.000-0.120)	0.708
B/Yamagata	12	11	0.041 (0.013-0.095)	0.031 (0.003-0.100)	0.902
Pre-vaccination $CD4^+$ TNF-α					
A/H1N1	14	12	0.003 (0.000-0.012)	0.000 (0.000-0.022)	0.282
A/H3N2	14	8	0.003 (0.000-0.007)	0.000 (0.000-0.005)	**0.040***
B/Victoria	13	6	0.001 (0.000-0.012)	0.001 (0.000-0.006)	0.817
B/Yamagata	12	3	0.002 (0.000-0.006)	0.000 (0.000-0.003)	0.389
Post-vaccination $CD4^+$ TNF-α					
A/H1N1	21	21	0.012 (0.000-0.044)	0.004 (0.000-0.150)	0.197
A/H3N2	20	18	0.010 (0.000-0.053)	0.009 (0.000-0.120)	0.568
B/Victoria	20	15	0.014 (0.000-0.033)	0.007 (0.000-0.120)	0.907
B/Yamagata	15	12	0.018 (0.005-0.053)	0.007 (0.000-0.220)	0.157
Pre-vaccination $CD4^+$ IL-2					
A/H1N1	13	14	0.003 (0.000-0.014)	0.001 (0.000-0.009)	0.554
A/H3N2	13	10	0.003 (0.000-0.016)	0.003 (0.000-0.013)	0.774
B/Victoria	11	8	0.000 (0.000-0.030)	0.000 (0.000-0.014)	0.600
B/Yamagata	9	5	0.003 (0.000-0.022)	0.004 (0.000-0.008)	1.000

	Study group, samples included	Control group, samples included	Study group: % of cells expressing cytokines	Control group: % of cells expressing cytokines	*p*-value
Post-vaccination CD4⁺ IL-2					
A/H1N1	21	20	0.018 (0.000-0.180)	0.008 (0.000-0.370)	0.531
A/H3N2	20	17	0.021 (0.000-0.200)	0.014 (0.000-0.320)	0.636
B/Victoria	19	15	0.023 (0.000-0.096)	0.018 (0.000-0.350)	0.876
B/Yamagata	15	13	0.030 (0.003-0.170)	0.011 (0.000-0.400)	0.369
Pre-vaccination CD4⁺ IL-13					
A/H1N1	9	6	0.003 (0.000-0.015)	0.004 (0.000-0.014)	1.000
A/H3N2	9	3	0.004 (0.000-0.007)	0.005 (0.000-0.007)	0.565
B/Victoria	9	0	0.001 (0.000-0.016)		
B/Yamagata	8	0	0.004 (0.000-0.009)		
Post-vaccination CD4⁺ IL-13					
A/H1N1	15	15	0.008 (0.000-0.016)	0.011 (0.000-0.027)	0.618
A/H3N2	15	13	0.012 (0.000-0.018)	0.006 (0.000-0.024)	0.729
B/Victoria	14	12	0.010 (0.000-0.020)	0.013 (0.000-0.023)	0.536
B/Yamagata	11	10	0.010 (0.003-0.015)	0.010 (0.000-0.020)	1.000
Pre-vaccination CD8⁺ IFN-γ					
A/H1N1	9	9	0.013 (0.000-0.029)	0.009 (0.000-0.029)	0.230
A/H3N2	9	7	0.005 (0.000-0.025)	0.007 (0.000-0.026)	0.831
B/Victoria	7	6	0.009 (0.000-0.024)	0.003 (0.000-0.011)	0.339
B/Yamagata	6	4	0.011 (0.002-0.023)	0.003 (0.000-0.020)	0.336
Post-vaccination CD8⁺ IFN-γ					
A/H1N1	15	21	0.020 (0.000-0.064)	0.010 (0.000-0.068)	0.228
A/H3N2	14	18	0.016 (0.000-0.041)	0.009 (0.000-0.045)	0.203
B/Victoria	13	15	0.018 (0.000-0.038)	0.017 (0.000-0.036)	0.982
B/Yamagata	11	11	0.019 (0.000-0.046)	0.026 (0.000-0.030)	0.948
Pre-vaccination CD8⁺ TNF-α					
A/H1N1	7	8	0.000 (0.000-0.010)	0.000 (0.000-0.005)	0.408
A/H3N2	7	6	0.000 (0.000-0.006)	0.000 (0.000-0.010)	0.931
B/Victoria	6	6	0.000 (0.000-0.004)	0.000 (0.000-0.003)	1.000
B/Yamagata	5	5	0.000 (0.000-0.008)	0.000 (0.000-0.005)	0.607

	Study group, samples included	Control group, samples included	Study group: % of cells expressing cytokines	Control group: % of cells expressing cytokines	*p*-value
Post-vaccination CD8⁺ TNF-α					
A/H1N1	21	22	0.003 (0.000-0.031)	0.001 (0.000-0.010)	0.485
A/H3N2	19	19	0.003 (0.000-0.018)	0.000 (0.000-0.013)	0.054
B/Victoria	19	16	0.005 (0.000-0.018)	0.003 (0.000-0.008)	0.284
B/Yamagata	15	11	0.006 (0.000-0.015)	0.002 (0.000-0.020)	0.218
Pre-vaccination CD8⁺ IL-2					
A/H1N1	6	4	0.000 (0.000-0.004)	0.000 (0.000-0.000)	0.540
A/H3N2	6	3	0.000 (0.000-0.003)	0.000 (0.000-0.000)	0.637
B/Victoria	6	2	0.002 (0.000-0.005)	0.000 (0.000-0.000)	0.340
B/Yamagata	5	2	0.000 (0.000-0.003)	0.000 (0.000-0.000)	0.469
Post-vaccination CD8⁺ IL-2					
A/H1N1	18	16	0.002 (0.000-0.011)	0.000 (0.000-0.012)	0.189
A/H3N2	18	14	0.002 (0.000-0.005)	0.001 (0.000-0.005)	0.356
B/Victoria	18	12	0.002 (0.000-0.011)	0.001 (0.000-0.007)	0.384
B/Yamagata	14	10	0.003 (0.000-0.006)	0.001 (0.000-0.003)	0.164
Pre-vaccination CD8⁺ IL-13					
A/H1N1	8	2	0.002 (0.000-0.013)	0.012 (0.007-0.016)	0.138
A/H3N2	8	2	0.004 (0.000-0.014)	0.005 (0.000-0.010)	1.000
B/Victoria	8	2	0.002 (0.000-0.020)	0.004 (0.000-0.008)	0.889
B/Yamagata	7	2	0.003 (0.000-0.007)	0.005 (0.005-0.006)	0.180
Post-vaccination CD8⁺ IL-13					
A/H1N1	11	11	0.006 (0.000-0.014)	0.003 (0.000-0.009)	0.087
A/H3N2	11	10	0.006 (0.000-0.014)	0.006 (0.000-0.015)	0.972
B/Victoria	10	8	0.003 (0.000-0.016)	0.006 (0.004-0.012)	0.283
B/Yamagata	9	7	0.006 (0.001-0.013)	0.005 (0.002-0.015)	0.916

*: The difference between the groups did not remain significant after correction for multiple comparisons with the Benjamini-Hochberg procedure.

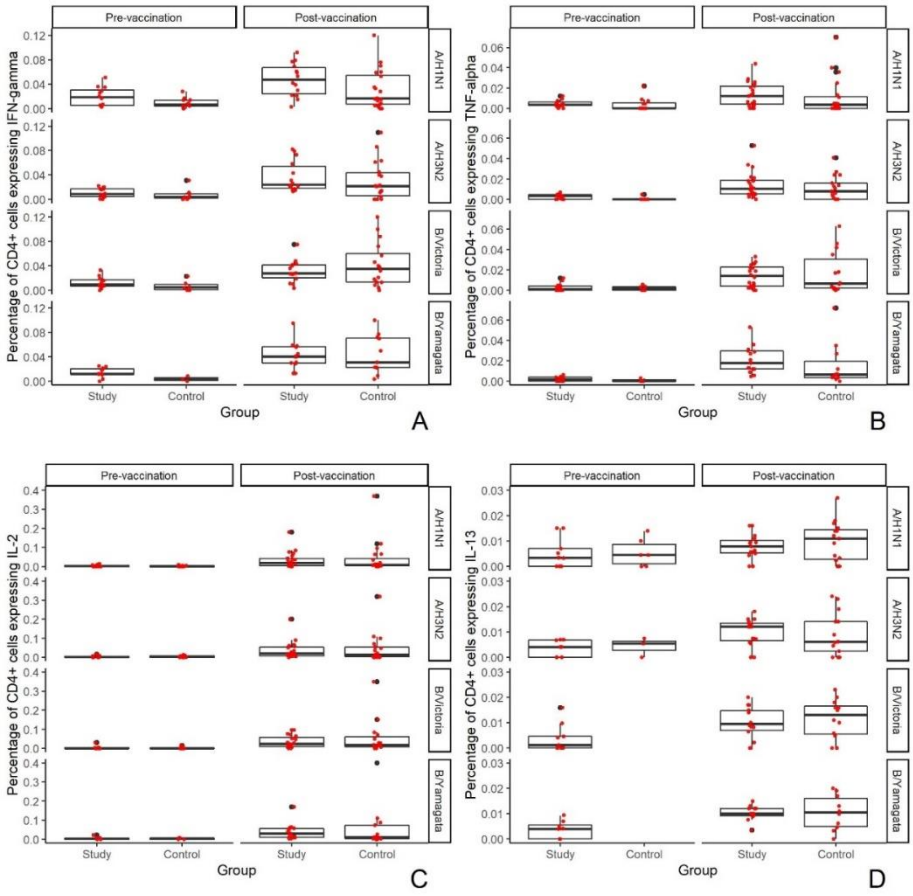

Figure 14. *T cell stimulation assay for CD4⁺ T cells.*

The boxplots show the percentage of pre- and post-vaccination CD4⁺ cells expressing A) IFN-γ, B) TNF-α, C) IL-2 and D) IL-13 in response to stimulation with influenza A/H1N1, A/H3N2, B/Victoria and B/Yamagata vaccine strains for adolescents with obesity (Study group) and adolescents with normal weight (Control group). The analysis of IL-13 expression did not include any pre-vaccination cells from the control group stimulated with influenza B/Victoria and B/Yamagata (D). The red points represent individual measurements. The boxplots show the median; first and third quartiles; with whiskers extending to +/- 1.5 * interquartile range; and outliers.

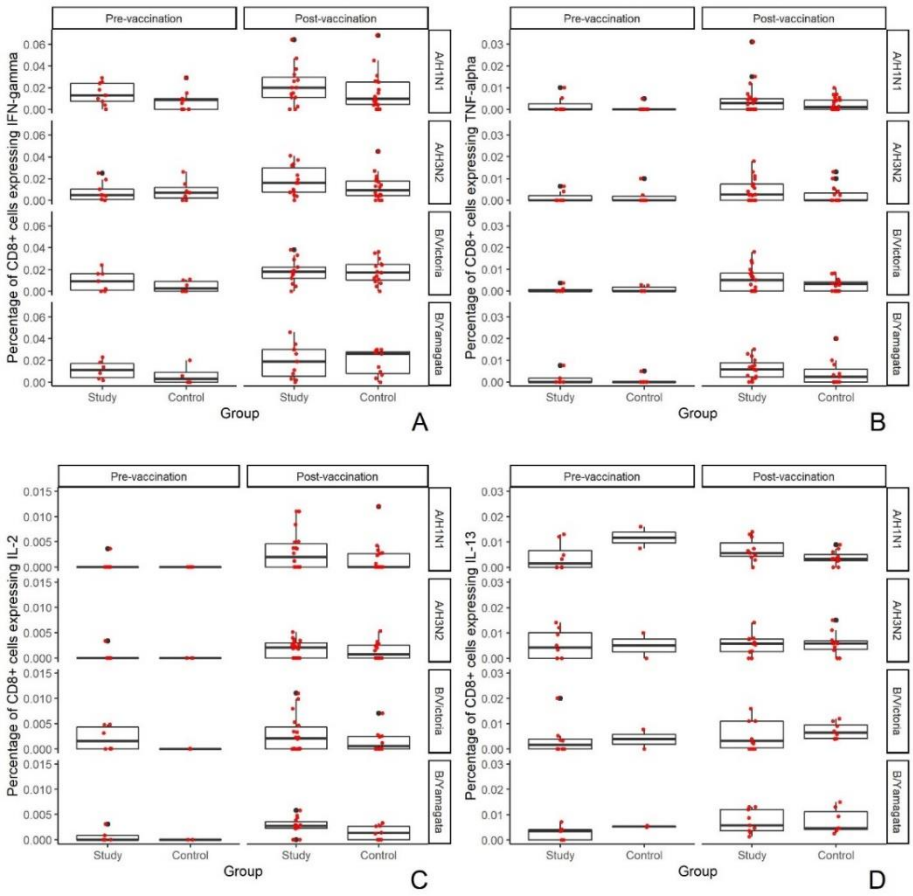

Figure 15. *T cell stimulation assay for CD8⁺ T cells.*

The boxplots show the percentage of pre- and post-vaccination CD8⁺ cells secreting A) IFN-γ, B) TNF-α, C) IL-2 and D) IL-13 in response to stimulation with influenza A/H1N1, A/H3N2, B/Victoria and B/Yamagata vaccine strains for adolescents with obesity (Study group) and adolescents with normal weight (Control group). A limited number of samples were included in some experiments. For example, only two samples were included in the pre-vaccination measurements of IL-13 for the control group (panel D). The red points represent individual measurements. The boxplots show the median; first and third quartiles; with whiskers extending to +/- 1.5 * interquartile range; and outliers.

Table 19. *T cell stimulation assay: Percentage of cells secreting cytokines in response to stimulation to the vaccine strains – study group, pre- vs post-vaccination.*

Comparison of percentage of cells from the study group secreting the measured cytokines in response to stimulation with the vaccine influenza strains, pre- vs post-vaccination. Samples were only included if paired measurements were available (pre- and post-vaccination). No significant differences remained after correction for multiple comparisons with the Benjamini-Hochberg procedure.

	No. of paired samples	Pre-vaccination % of cells expressing cytokines	Post-vaccination % of cells expressing cytokines	*p*-value
$CD4^+$ IFN-γ				
A/H1N1	9	0.026 (0.003-0.051)	0.059 (0.003-0.079)	0.025
A/H3N2	9	0.016 (0.000-0.022)	0.022 (0.013-0.082)	0.012
B/Victoria	9	0.013 (0.000-0.033)	0.027 (0.003-0.048)	0.041
B/Yamagata	7	0.018 (0.011-0.025)	0.031 (0.013-0.058)	0.008
$CD4^+$ TNF-α				
A/H1N1	12	0.004 (0.000-0.012)	0.013 (0.000-0.027)	0.029
A/H3N2	11	0.003 (0.000-0.007)	0.011 (0.000-0.034)	0.010
B/Victoria	10	0.000 (0.000-0.012)	0.017 (0.000-0.025)	0.033
B/Yamagata	8	0.003 (0.000-0.006)	0.017 (0.005-0.031)	0.008
$CD4^+$ IL-2				
A/H1N1	10	0.004 (0.000-0.014)	0.020 (0.000-0.180)	0.021
A/H3N2	9	0.003 (0.000-0.016)	0.028 (0.008-0.200)	0.004
B/Victoria	7	0.004 (0.000-0.030)	0.038 (0.012-0.096)	0.016
B/Yamagata	6	0.005 (0.000-0.022)	0.044 (0.014-0.170)	0.031
$CD4^+$ IL-13				
A/H1N1	4	0.002 (0.000-0.015)	0.006 (0.004-0.016)	0.554
A/H3N2	4	0.005 (0.000-0.007)	0.009 (0.000-0.015)	0.144
B/Victoria	3	0.000 (0.000-0.016)	0.008 (0.002-0.020)	0.113
B/Yamagata	2	0.003 (0.000-0.007)	0.014 (0.012-0.015)	0.124
$CD8^+$ IFN-γ				
A/H1N1	7	0.013 (0.000-0.025)	0.014 (0.003-0.047)	0.150
A/H3N2	6	0.008 (0.000-0.025)	0.012 (0.004-0.041)	0.080
B/Victoria	5	0.009 (0.000-0.016)	0.022 (0.000-0.038)	0.044
B/Yamagata	4	0.016 (0.003-0.023)	0.028 (0.000-0.030)	0.145
$CD8^+$ TNF-α				
A/H1N1	5	0.000 (0.000-0.010)	0.002 (0.000-0.004)	1.000
A/H3N2	4	0.000 (0.000-0.004)	0.001 (0.000-0.003)	1.000
B/Victoria	4	0.000 (0.000-0.004)	0.000 (0.000-0.014)	1.000
B/Yamagata	3	0.000 (0.000-0.008)	0.007 (0.003-0.013)	0.050
$CD8^+$ IL-2				
A/H1N1	5	0.000 (0.000-0.004)	0.003 (0.000-0.011)	0.361
A/H3N2	5	0.000 (0.000-0.003)	0.003 (0.000-0.005)	0.438
B/Victoria	5	0.003 (0.000-0.005)	0.000 (0.000-0.011)	1.000
B/Yamagata	3	0.000 (0.000-0.000)	0.003 (0.002-0.006)	0.075
$CD8^+$ IL-13				
A/H1N1	3	0.003 (0.000-0.012)	0.006 (0.000-0.007)	0.705
A/H3N2	3	0.009 (0.005-0.012)	0.007 (0.003-0.008)	0.492
B/Victoria	3	0.000 (0.000-0.004)	0.004 (0.000-0.011)	0.218
B/Yamagata	2	0.005 (0.003-0.007)	0.006 (0.003-0.009)	1.000

Table 20. *T cell stimulation assay: Percentage of cells secreting cytokines in response to stimulation to the vaccine strains – control group, pre- vs post-vaccination.*

Comparison of percentage of cells from the control group secreting the measured cytokines in response to stimulation with the vaccine influenza strains, pre- vs post-vaccination. Samples were only included if paired measurements were available (pre- and post-vaccination). Paired samples were only available for one participant for $CD4^+$ IL-13 A/H3N2 and none for $CD4^+$ IL-13 B/Victoria and B/Yamagata. No significant differences remained after correction for multiple comparisons with the Benjamini-Hochberg procedure.

	No. of paired samples	**Pre-vaccination % of cells expressing cytokines**	**Post-vaccination % of cells expressing cytokines**	***p*-value**
$CD4^+$ IFN-γ				
A/H1N1	9	0.006 (0.000-0.028)	0.016 (0.000-0.120)	0.183
A/H3N2	6	0.006 (0.000-0.031)	0.031 (0.004-0.086)	0.156
B/Victoria	5	0.004 (0.000-0.011)	0.046 (0.013-0.120)	0.063
B/Yamagata	3	0.003 (0.000-0.008)	0.023 (0.022-0.050)	0.250
$CD4^+$ TNF-α				
A/H1N1	11	0.000 (0.000-0.022)	0.004 (0.000-0.150)	0.058
A/H3N2	7	0.000 (0.000-0.005)	0.011 (0.000-0.024)	0.047
B/Victoria	5	0.000 (0.000-0.004)	0.002 (0.001-0.017)	0.188
B/Yamagata	3	0.000 (0.000-0.003)	0.004 (0.002-0.007)	0.256
$CD4^+$ IL-2				
A/H1N1	11	0.002 (0.000-0.009)	0.005 (0.000-0.370)	0.042
A/H3N2	7	0.004 (0.000-0.013)	0.013 (0.000-0.053)	0.128
B/Victoria	7	0.000 (0.000-0.014)	0.017 (0.005-0.029)	0.017
B/Yamagata	4	0.005 (0.000-0.008)	0.007 (0.000-0.025)	0.423
$CD4^+$ IL-13				
A/H1N1	4	0.002 (0.000-0.014)	0.011 (0.000-0.027)	0.385
A/H3N2	1	0.005	0.024	
B/Victoria	0			
B/Yamagata	0			
$CD8^+$ IFN-γ				
A/H1N1	8	0.009 (0.000-0.029)	0.011 (0.000-0.031)	0.368
A/H3N2	6	0.006 (0.000-0.026)	0.006 (0.000-0.045)	0.605
B/Victoria	5	0.000 (0.000-0.011)	0.017 (0.009-0.036)	0.022
B/Yamagata	3	0.000 (0.000-0.006)	0.014 (0.007-0.027)	0.083
$CD8^+$ TNF-α				
A/H1N1	6	0.000 (0.000-0.005)	0.003 (0.000-0.007)	0.181
A/H3N2	5	0.000 (0.000-0.010)	0.000 (0.000-0.005)	1.000
B/Victoria	4	0.000 (0.000-0.003)	0.003 (0.000-0.005)	0.375
B/Yamagata	3	0.000 (0.000-0.000)	0.000 (0.000-0.003)	1.000
$CD8^+$ IL-2				
A/H1N1	3	0.000 (0.000-0.000)	0.000 (0.000-0.002)	1.000
A/H3N2	3	0.000 (0.000-0.000)	0.000 (0.000-0.002)	1.000
B/Victoria	2	0.000 (0.000-0.000)	0.002 (0.001-0.002)	0.500
B/Yamagata	2	0.000 (0.000-0.000)	0.002 (0.000-0.003)	1.000
$CD8^+$ IL-13				
A/H1N1	1	0.007	0.004	
A/H3N2	1	0.000	0.011	
B/Victoria	1	0.000	0.007	
B/Yamagata	1	0.006	0.005	

4.3.4 Exclusion of non-normal weight participants from the control group

Six participants in the control group did not have normal weight, according to definition, as presumed at recruitment. Four were underweight (BMI 15.4, 15.4, 16.0 and 16.5 kg/m^2, respectively) and two were overweight (BMI 22.4 and 23.0 kg/m^2, respectively). After excluding non-normal weight participants from the control group, the post-vaccination titres against influenza B/Victoria were higher, so the difference between the study group and the control group did not remain significant (data from Table 14). The proportion of CD4$^+$ cells secreting TNF-α in response to influenza A/H1N1 pre-vaccination also increased by excluding non-normal weight participants from the control group, so the difference between the two groups was no longer significant (data from Table 18). No other significant changes were observed.

4.4 Study IV – FluRisk: Influenza vaccine uptake and burden of influenza illness in pregnant women and their infants

4.4.1 Influenza vaccine uptake in pregnant women

The study included 24,577 pregnancies among 19,565 women. The 20-week ultrasounds per woman during the study period ranged from one to four (a median of one). Women attending the 20-week ultrasounds in May, June and July were excluded, as antenatal care appointments are unlikely to have coincided with the influenza vaccination period. The number of pregnant women included in the study each season ranged from 2,265 to 2,640 (median 2,446) (Figure 16A).

The overall vaccine uptake for the ten influenza seasons was 19.1% (vaccines administered in 4,686/24,577 pregnancies). The lowest vaccine uptake was in 2011-2012, when 6.2% of the pregnant women in the cohort were vaccinated (Figure 16B). The vaccine uptake increased over the period and reached 37.5% in 2019-2020 (Figure 16B). Influenza vaccines were most often administered in the second trimester of pregnancy (47.4%), 21.1% were administered in the third trimester, 20.9% in the first trimester and 10.7% likely prior to conception. Most influenza vaccines were administered in October (44.6%), November (22.4%) and September (12.2%) (Figure 17).

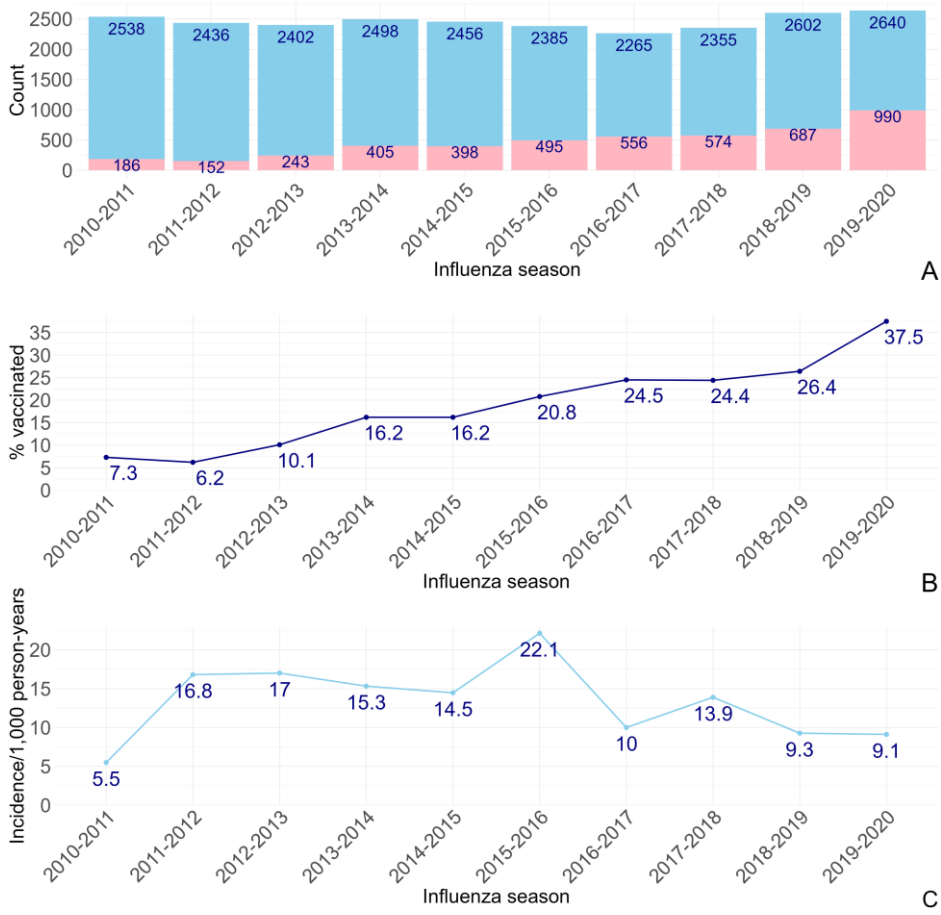

Figure 16. *Pregnant women, influenza vaccinations, and influenza/ILI diagnoses each influenza season, 2010-2020.*

A: The figure shows the number of women attending 20-week ultrasounds at Landspitali University Hospital in August-April each influenza season (blue bars) and the number of them that were vaccinated each influenza season (superimposed pink bars). The numbers in the blue bars represent the total number of women in the cohort in the respective seasons, and the numbers in the pink bars the numbers of vaccinated women.

B: The percentage of pregnant women in the cohort vaccinated each influenza season.

C: The incidence rate of influenza/ILI diagnoses per 1,000-person years each influenza season during the study period.

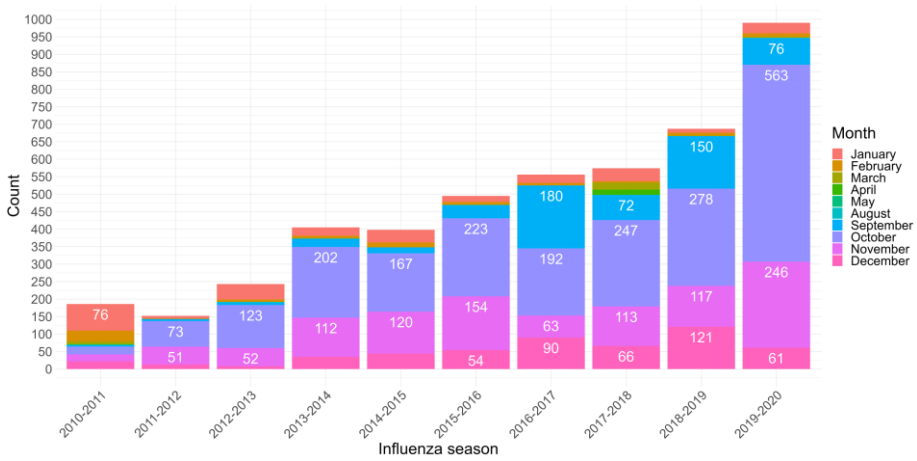

Figure 17. *Vaccination month of pregnant women in the ten influenza seasons 2010-2020.*

The figure shows the number of vaccinations by month and influenza season. Most women were vaccinated in the autumn.

4.4.2 Disease burden of influenza in pregnant women

A total of 230 cases of influenza/ILI were diagnosed in pregnant women in the ten influenza seasons, of which 217 (94%) were laboratory-confirmed influenza. The proportion of laboratory-confirmed influenza cases ranged from 89.2% to 100% by season. Influenza/ILI cases per season ranged from ten to 37 (Tables 21 and 22). The incidence rate of influenza was lowest at 5.5 cases per 1,000 person-years in 2010-2011 and highest at 22.1 cases per 1,000 person-years in 2015-2016 (Figure 16, Table 22). Of the 230 influenza/ILI diagnoses in the cohort over the ten seasons, 19 (8.3%) were diagnosed in vaccinated women, of which 16 were laboratory confirmed. No woman was diagnosed with influenza/ILI before receiving a vaccination in the respective influenza season. Influenza/ILI was diagnosed 31-180 days after influenza vaccinations. There was not a significant difference in the proportion of women diagnosed with influenza/ILI by the trimester of vaccination; 0.3% (3/978) of women vaccinated in the first trimester were diagnosed with influenza/ILI, 0.5% (11/2219) of women vaccinated in the second trimester, 0.10% (1/988) of women vaccinated in the third trimester and 0.8% (4/501) of women vaccinated prior to conception (p = 0.14).

The crude incidence of influenza/ILI ranged from 3.94 to 15.51 cases per 1,000 pregnant women, 0.0-6.58 per 1,000 vaccinated pregnant women and 4.25-18.52 per 1,000 unvaccinated pregnant women (Table 21). The

incidence rate for unvaccinated women was highest in 2015-2016 (26.87/1,000 person-years), but for vaccinated women in 2011-2012 (8.96/1,000 person-years) (Figure 18, Table 22). The incidence rate was higher for unvaccinated women than for vaccinated women in all ten influenza seasons, and the IRR was below one for all seasons, although only 2015-2016 had a confidence interval that did not overlap one (Table 22). The IRRs were not heterogenous for the ten seasons (χ^2 test of heterogeneity: p = 0.98). The combined Mantel-Haenszel IRR for the ten influenza seasons was 0.36 (95% CI 0.22-0.58), which suggests an overall protection of 64% for the ten influenza seasons (Table 22). The estimated vaccine effectiveness for individual seasons ranged from 34-100% (Figure 19).

Seven influenza/ILI-associated hospitalisations occurred in the ten influenza seasons, all due to laboratory-confirmed influenza in unvaccinated pregnant women. One influenza-associated intensive care unit admission was registered for a pregnant woman in the study period, but no influenza-associated deaths were registered for the pregnant women in the cohort.

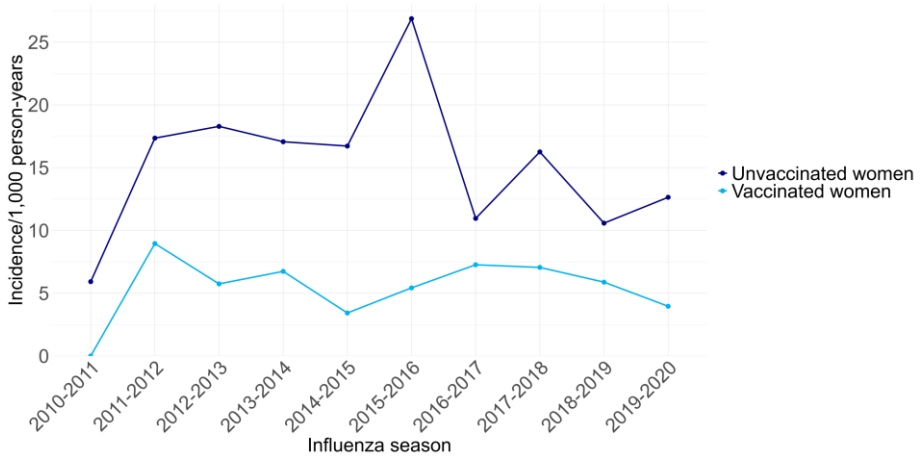

Figure 18. *The incidence rate of influenza/ILI diagnoses among pregnant women for the ten influenza seasons by influenza vaccination status.*

The graph shows the incidence rate of influenza/ILI in unvaccinated women in the cohort (navy-blue line) and vaccinated women in the cohort (sky-blue line) for each influenza season. The incidence rate is presented as the number of cases per 1,000 person-years.

Table 21. *The crude incidence of influenza/ILI in vaccinated and unvaccinated pregnant women, stratified by influenza season.*

The incidence is presented as cases per 1,000 pregnant women.

Influenza season	n cases, vaccinated	n cases, unvaccinated	Crude incidence, total	Crude incidence, vaccinated	Crude incidence, unvaccinated
2010-2011	0	10	3.94	0	4.25
2011-2012	1	28	11.90	6.58	12.26
2012-2013	1	28	12.07	4.12	12.97
2013-2014	2	25	10.81	4.94	11.94
2014-2015	1	24	10.18	2.51	11.66
2015-2016	2	35	15.51	4.04	18.52
2016-2017	3	13	7.06	5.40	7.61
2017-2018	3	20	9.77	5.23	11.23
2018-2019	3	14	6.53	4.37	7.31
2019-2020	3	14	6.44	3.03	8.48

Table 22. *Influenza/ILI incidence rates (IR) and incidence rate ratios (IRR) for vaccinated and unvaccinated pregnant women, stratified by influenza season.*

The table shows the number of influenza/ILI cases diagnosed in pregnant women and the incidence rate and incidence rate ratio by vaccination status, stratified by influenza season. The incidence rate is presented per 1,000 person-years. The incidence rate ratio is presented for vaccinated vs unvaccinated pregnant women. The incidence rate ratio (IRR) was <1 for each of the ten influenza seasons, but the season 2015-2016 was the only one with a confidence interval that did not overlap 1 (in bold).

Influenza season	n cases, vaccinated	n cases, unvaccinated	IR, total	IR, vaccinated	IR, unvaccinated	IRR (95% CI)
2010-2011	0	10	5.49	0	5.92	0 (0.00-5.69)
2011-2012	1	28	16.81	8.96	17.35	0.52 (0.01-3.12)
2012-2013	1	28	17.01	5.75	18.29	0.31 (0.01-1.90)
2013-2014	2	25	15.33	6.74	17.07	0.39 (0.05-1.58)
2014-2015	1	24	14.48	3.43	16.72	0.20 (0.005-1.26)
2015-2016	**2**	**35**	**22.14**	**5.43**	**26.87**	**0.20 (0.02-0.79)**
2016-2017	3	13	10.01	7.27	10.96	0.66 (0.12-2.41)
2017-2018	3	20	13.89	7.06	16.26	0.43 (0.08-1.46)
2018-2019	3	14	9.28	5.88	10.58	0.56 (0.10-1.99)
2019-2020	3	14	9.12	3.96	12.64	0.31 (0.06-1.12)
χ^2 test of heterogeneity: $p = 0.98$ Mantel-Haenszel combined IRR estimate: 0.36 (95% CI 0.22-0.58)						

IR: Incidence rate (cases per 1000-person years).

IRR: Incidence rate ratio, vaccinated vs unvaccinated women.

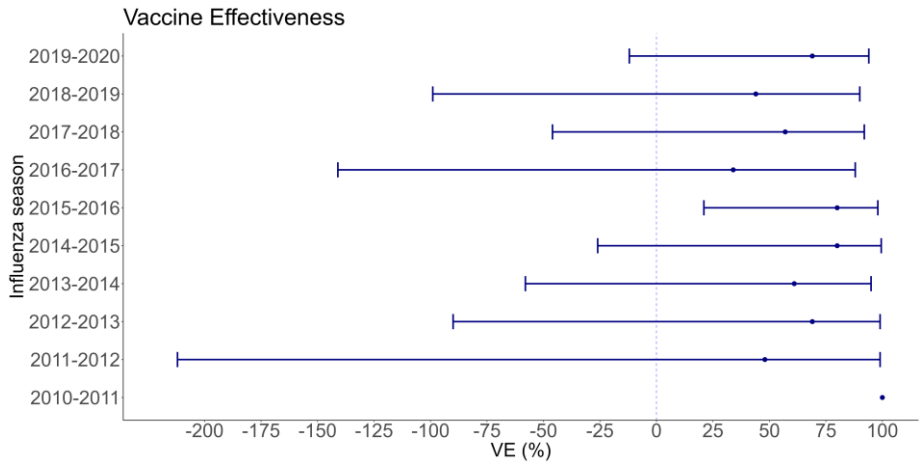

Figure 19. *Estimated influenza vaccine effectiveness for pregnant women.*
The figure shows the estimated influenza vaccine effectiveness against influenza/ILI in the ten influenza seasons. No influenza/ILI was diagnosed in the vaccinated group in 2010-2011, resulting an an estimated VE of 100% (95% CI -469% to 100%). The large confidence interval for 2010-2011 is omitted from the figure for clarity.

4.4.3 Disease burden of influenza in infants

No infants were registered for 4.3% of pregnancies included in the cohort of pregnant women (1,046/24,577). Maternal influenza vaccinations were registered for 15.3% (160/1,046) of those. In total, 23,906 infants born after a pregnancy included in the cohort of pregnant women were included in the cohort of infants, of which 51.5% were boys (12,308) and 48.5% were girls (11,596). The sex was not recorded for two infants. Nineteen per cent of infants were born to mothers vaccinated >14 days before birth (4,577/23,906).

A total of 175 influenza/ILI cases were diagnosed among infants under the age of 12 months, of which 170 (97.1%) were laboratory confirmed. Ninety-two cases were diagnosed in female infants (52.6%) and 83 in male infants (47.4%) (p = 0.32). Influenza/ILI was most often diagnosed in February (33.7%), followed by March (27.4%) and January (21.7%) (Figure 20). The median age at influenza/ILI diagnosis was 8.4 months (range 0.16-11.9 months). Fifteen per cent (27/175) of infants diagnosed with influenza/ILI in the first year of life were born to vaccinated mothers. The median age at diagnosis of influenza/ILI in infants born to vaccinated mothers was 9.3 months (range 1.4-11.7 months) and 8.2 months (range 0.16-11.9 months)

for infants born to unvaccinated mothers (p = 0.12). Only 2/175 (1.14%) of infants diagnosed with influenza/ILI had mothers also diagnosed with influenza, but in both cases, some months separated the influenza diagnoses of the infant and the mother. The relative number of influenza/ILI cases in infants did not differ significantly by the trimester of maternal vaccination; 0.23% (1/436) of infants born to mothers vaccinated prior to conception were diagnosed with influenza/ILI, 0.31% (3/979) of infants born to women vaccinated in the first trimester, 0.81% (18/2218) of infants born to women vaccinated in the second trimester and 0.53% (5/944) of infants born to women vaccinated in the third trimester (p = 0.32).

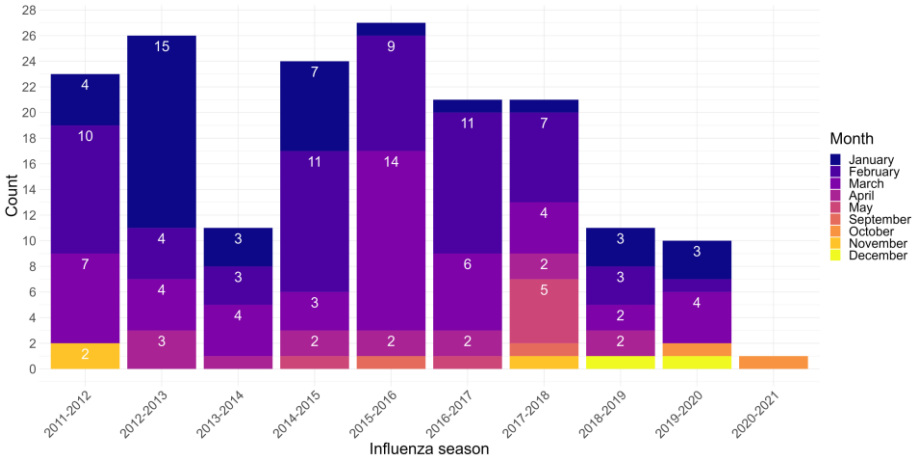

Figure 20. *The number of influenza/ILI diagnoses in infants in their first year of life, by month and influenza season.*
No influenza/ILI was diagnosed among infants in the cohort in the season 2010-2011 and it is therefore excluded from the figure.

A quarter (43/175) of all infant influenza/ILI diagnoses were in the same season as the mother should have been offered a vaccination. Six (14%) of the infants diagnosed with influenza/ILI in the season of maternal vaccination were born to vaccinated mothers. The six influenza/ILI cases were all diagnosed in two influenza seasons, 2016-2017 and 2017-2018 (Table 23, Figure 21A), but none in the other eight seasons. The crude incidence for influenza/ILI among infants in the season of maternal vaccination ranged from 0 to 9.15 per 1,000 infants (Table 23) and the incidence rate ranged from 0 to 40.52 per 1,000 person-years (Table 24). The IRRs were <1 for nine out of ten seasons, but 1.01 in 2016-2017 (Table 24). The IRRs over the ten seasons were not heterogenous (p = 0.92), and the combined Mantel-

Haenszel IRR was 0.40 (95% CI 0.17-0.97) (Table 24). The vaccine effectiveness for individual seasons was estimated to range from -1% to 100%. Seventeen infants, all born to unvaccinated mothers, were hospitalised with influenza/ILI in the season of maternal vaccination.

Influenza/ILI was diagnosed in 54 infants <6 months of age during the ten influenza seasons, of which six (11.1%) were born to vaccinated mothers. The incidence rate for infants born to vaccinated mothers was highest in 2016-2017 and in 2017-2018 for infants born to unvaccinated mothers (Figure 21B). The IRRs stratified by influenza season are shown in Table 24. The IRRs ranged from 0 to 1.79 but was only >1 in the season 2016-2017. The IRRs were not heterogenous (p = 0.71). The estimated combined Mantel-Haenszel IRR was 0.51 (95% CI 0.22-1.21), which suggests probable protection of maternal vaccination for infants <6 months of age. Eighteen infants <6 months of age had influenza/ILI-associated hospitalisations. None were born to vaccinated mothers (p = 0.16).

For infants <12 months of age, the incidence rate ranged from 0 to 13.41 per 1,000 person-years, and the IRRs from 0 to 1.45 (Table 24) and were not heterogenous (p = 0.97). The combined Mantel-Haenszel IRR estimate for the ten seasons was 0.91 (95% CI 0.59-1.38). The incidence rates for infants <12 months of age born to vaccinated mothers and unvaccinated mothers are shown in Figure 21C.

Over the ten influenza seasons, a total of 21 infants <12 months of age had influenza/ILI-associated hospitalisations (12% of infants diagnosed with influenza/ILI) (Table 25). The median age at hospitalisation was 38 days (range 18-319 days). The majority were admitted in the first six months of life (18/21, 85.7%). The incidence of hospitalisations for infants <12 months and <6 months of age, by influenza season, is shown in Table 25. The incidence for infants <12 months of age ranged from 0 to 1.17 hospitalisations per 1,000 infants. Hospitalisations were most common in 2014-2015 when five infants <12 months of age were hospitalised. Only one hospitalised infant was born to a vaccinated mother, which was admitted at 10 months of age, in the season following the mother's vaccination (OR 0.25, 95% CI 0.006-1.69, p = 0.21). The median duration of hospitalisation was two days (range 1-4 days). No infants were admitted to a NICU/ICU with influenza/ILI. Ten infants (47.6% of the hospitalised infants) were treated with oseltamivir during the hospitalisation. Five of the 21 hospitalised infants (23.8%) had potential complications from influenza: two were diagnosed with acute otitis media, one with pneumonia, one with acute bronchiolitis, one with acute laryngotracheitis, one with transient neonatal neutropenia and one with acute tubulointerstitial nephritis.

Table 23. *Crude incidence of influenza/ILI diagnoses among infants in the season of maternal vaccination, infants <6 months of age, and infants <12 months of age.*

The incidence is presented as the number of cases per 1,000 infants. Infants were considered at risk in both influenza seasons that overlapped with the first six and 12 months of life, respectively.

Influenza season	n cases, mother vaccinated	n cases, mother not vaccinated	Crude incidence	Crude incidence, infants born to vaccinated mothers	Crude incidence, infants born to unvaccinated mothers
Season of maternal vaccination					
2010-2011	0	0	0	0	0
2011-2012	0	3	2.11	0	2.28
2012-2013	0	3	2.10	0	2.34
2013-2014	0	0	0	0	0
2014-2015	0	5	3.58	0	4.40
2015-2016	0	7	5.13	0	6.88
2016-2017	4	8	9.15	10.23	8.70
2017-2018	2	7	6.68	5.21	7.27
2018-2019	0	2	1.33	0	1.94
2019-2020	0	2	1.29	0	2.57
Infants <6 months of age					
2010-2011	0	0	0	0	0
2011-2012	0	3	0.85	0	0.93
2012-2013	0	5	1.45	0	1.57
2013-2014	0	0	0	0	0
2014-2015	0	9	2.57	0	3.09
2015-2016	0	8	2.34	0	2.91
2016-2017	4	8	3.65	5.23	3.17
2017-2018	2	8	3.12	2.48	3.33
2018-2019	0	4	1.14	0	1.55
2019-2020	0	3	0.80	0	1.22
2020-2021*	0	0	0	0	0
Infants <12 months of age					
2010-2011	0	0	0	0	0
2011-2012	0	23	5.85	0	6.31
2012-2013	2	24	6.14	6.21	6.13
2013-2014	1	10	2.59	1.84	2.69
2014-2015	3	21	5.59	4.29	5.85
2015-2016	4	23	6.38	5.03	6.70
2016-2017	6	15	5.12	6.47	4.72
2017-2018	6	15	5.23	6.05	4.96
2018-2019	2	9	2.59	1.80	2.88
2019-2020	2	8	2.20	1.32	2.63
2020-2021*	1	0	0.39	1.01	0

***** The study included influenza/ILI cases in infants up to the age of one year, therefore, the follow-up for infants extends to the season 2020-2021.

Table 24. *Influenza/ILI cases, incidence rates and incidence rate ratios for infants in the season of maternal vaccination, for infants <6 months of age and infants <12 months of age.*
The incidence rate is presented as the number of cases per 1,000 person-years.
IR: Incidence rate. IRR: Incidence rate ratio.

Influenza season	n cases, mother vaccinated	n cases, mother not vaccinated	IR, total	IR, infants born to vaccinated women	IR, infants born to unvaccinated women	IRR (95% CI)
Season of maternal vaccination						
2010-2011	0	0	0	0	0	-
2011-2012	0	3	9.12	0	9.98	0 (0-25.70)
2012-2013	0	3	9.17	0	10.13	0 (0-23.11)
2013-2014	0	0	0	0	0	-
2014-2015	0	5	15.91	0	19.64	0 (0-4.65)
2015-2016	0	7	22.50	0	30.95	0 (0-1.85)
2016-2017	4	8	40.19	40.52	40.02	1.01 (0.22-3.78)
2017-2018	2	7	29.85	21.76	33.41	0.65 (0.07-3.42)
2018-2019	0	2	5.88	0	9.18	0 (0-9.45)
2019-2020	0	2	5.57	0	13.06	0 (0-3.95)
χ^2 test of heterogeneity: $p = 0.92$ Mantel-Haenszel combined IRR estimate: 0.40 (95% CI 0.17-0.97)						
Infants <6 months of age						
2010-2011	0	0	0	0	0	-
2011-2012	0	3	3.14	0	3.42	0 (0.00-26.60)
2012-2013	0	5	5.28	0	5.66	0 (0.00-15.25)
2013-2014	0	0	0	0	0	-
2014-2015	0	9	9.37	0	11.10	0 (0.00-2.74)
2015-2016	0	8	8.38	0	10.31	0 (0.00-2.55)
2016-2017	4	8	13.13	20.06	11.20	1.79 (0.39-6.69)
2017-2018	2	8	11.42	9.84	11.90	0.83 (0.09-4.14)
2018-2019	0	4	4.19	0	5.63	0 (0.00-4.42)
2019-2020	0	3	2.90	0	4.35	0 (0.00-4.83)
2020-2021*	0	0	0	0	0	-
χ^2 test of heterogeneity: $p = 0.71$ Mantel-Haenszel combined IRR estimate: 0.51 (95% CI 0.22-1.21)						
Infants <12 months of age						
2010-2011	0	0	0	0	0	-
2011-2012	0	23	11.30	0	12.26	0 (0-2.03)
2012-2013	2	24	13.02	15.30	12.86	1.19 (0.14-4.79)
2013-2014	1	10	5.53	4.27	5.70	0.75 (0.02-5.27)
2014-2015	3	21	11.74	9.10	12.24	0.74 (0.14-2.49)
2015-2016	4	23	13.41	11.17	13.89	0.80 (0.20-2.35)
2016-2017	6	15	10.81	14.27	9.85	1.45 (0.46-3.95)
2017-2018	6	15	11.33	13.43	10.66	1.26 (0.40-3.44)
2018-2019	2	9	5.57	4.03	6.08	0.66 (0.07-3.21)
2019-2020	2	8	4.61	3.08	5.27	0.58 (0.06-2.93)
2020-2021*	1	0	0.69	1.82	0	-
χ^2 test of heterogeneity: $p = 0.97$ Mantel-Haenszel combined IRR estimate: 0.91 (95% CI 0.59-1.38)						

***** The study included influenza/ILI cases in infants up to the age of one year, therefore, the follow-up for infants extends to the season 2020-2021.

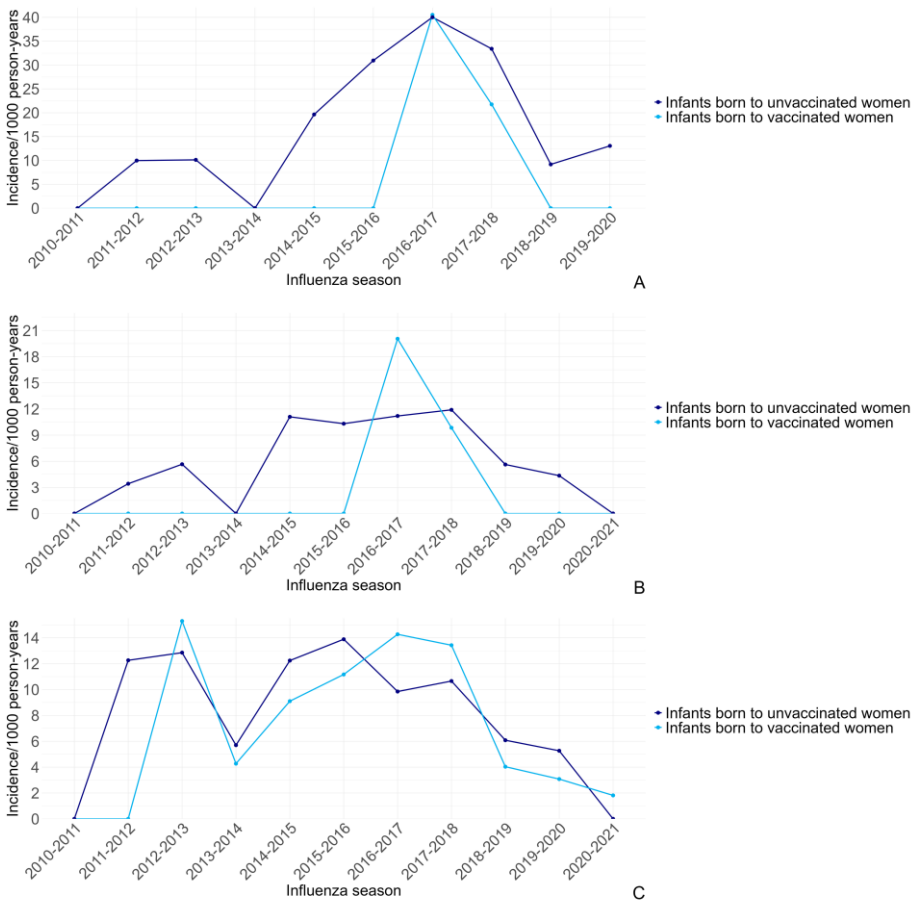

Figure 21. *The incidence rates of influenza/ILI by influenza season and maternal vaccination status for A: infants in the season of maternal vaccination, B: infants <6 months of age, and C: infants <12 months of age.*

The incidence rate for infants born to unvaccinated mothers is shown with a navy-blue line, and for infants born to vaccinated mothers with a sky-blue line. Note that the y-axes of the three panels have different scales and that the x-axes on panels B and C include the season 2020-2021.

Table 25. *The incidence of influenza/ILI-associated hospitalisations per 1,000 infants <6 months and <12 months of age.*

Influenza season	Hospitalisations per 1,000 infants <6 months of age	Hospitalisations per 1,000 infants <12 months of age
2010-2011	0	0
2011-2012	0.57	0.51
2012-2013	0.29	0.47
2013-2014	0	0.24
2014-2015	1.43	1.17
2015-2016	0.29	0.24
2016-2017	0.91	0.73
2017-2018	0.31	0.50
2018-2019	0.57	0.47
2019-2020	0.80	0.66
2020-2021	0	0

Antibiotics were prescribed for 52 infants (29.7%) in the four weeks following an influenza/ILI diagnosis. Antibiotics were prescribed to 11 infants born to vaccinated mothers (40.7% of infants diagnosed with influenza/ILI that were born to vaccinated mothers) and 41 infants born to unvaccinated mothers (27.7% of infants diagnosed with influenza/ILI that were born to unvaccinated mothers) (p = 0.26). The median age at the time of antibiotic treatment was 302 days (range 22-377 days). Six infants were treated with antibiotics following an influenza/ILI diagnosis in the first six months of life; none were born to a vaccinated mother (p = 0.59). The number of infants prescribed antibiotics ranged from zero to nine each season, with a median of five infants prescribed antibiotics per season. Twelve infants aged 25-325 days received prescriptions for oseltamivir in the ten influenza seasons.

Influenza-associated deaths were not registered for any infant in the cohort.

5 Discussions

5.1 Main findings

This thesis focuses on three vaccine-preventable pathogens: rotavirus, *Neisseria meningitidis*, and influenza. Vaccinations against these pathogens have public health importance, due to their prevalence or disease severity. The main findings of studies I-IV are summarised here:

- Rotavirus was the main cause of acute gastroenteritis in young children leading to emergency department visits. The disease burden of RVAGE was significant, as rotavirus causes more severe infections than other common enteropathogenic viruses. Children with RVAGE were more likely to require treatment and hospital admissions than children with non-RV AGE. RVAGE led to substantial parental absence from work, which was a major factor in the societal cost of RVAGE. The introduction of rotavirus vaccinations to the Icelandic immunisation schedule would be cost-effective (Study I).
- Colonisation with *Neisseria meningitidis* was uncommon in Icelandic children, adolescents, and young adults, with no pre-school children carrying meningococci, 0.5% of adolescents and 6.5% of young adults. Most carried non-groupable meningococci, but of capsulated meningococci, MenB was the most common. No MenC were detected. The longest duration of carriage (from the first positive to the last positive swab) was 21 months. All carriers carried the same meningococcal group for the duration of their carriage. WGS revealed close relatedness between strains from the same carrier at different sampling time points (Study II).
- Early humoral and cellular immune responses to influenza vaccinations were similar between adolescents with obesity and adolescents with normal weight. Seroconversion occurred for 80-100% of participants in both groups for the different influenza vaccine strains, and seroprotective titres were reached post-vaccination for 97-100% of participants. The two groups had similar post-vaccination cellular responses (Study III).
- The influenza vaccine uptake among pregnant women was suboptimal in the influenza seasons 2010-2020, although it increased from 6.2%

in 2011-2012 to 37.5% in 2019-2020. Influenza vaccinations in pregnancy protect pregnant women and their infants against influenza/ILI in the season of vaccination and provide probable protection for infants <6 months of age (Study IV).

5.2 Study I - RICE: Burden of rotavirus disease in young children in Iceland

In this thesis, the first prospective study on the burden of AGE and RVAGE in Iceland is presented. Our findings support what has previously been reported, that rotavirus is a common cause of AGE in young children and that the burden caused by rotavirus on families and health care systems is considerable.[34,37,45,88]

5.2.1 Acute gastroenteritis leading to emergency department visits

Rotavirus was the main causative pathogen in AGE episodes leading to ED visits, followed by adenovirus and norovirus. Bacterial infections were few. Rotavirus is frequently reported as the most common virus in AGE.[33-35,37] Unsurprisingly, most AGE episodes leading to an ED visit were severe or moderately severe according to the Vesikari score.

Co-infections occurred in 19.4% of AGE episodes, which is similar to what was reported in one study of children hospitalised with AGE[34] but higher than reported in another.[35] The difference in proportions of co-infections is likely partially explained by different study designs with regard to the viruses included.[34,35,42] Twenty-four per cent of RVAGE were co-infections, whereas half of the adenovirus cases were co-infections and almost three-quarters (30/41) of cases with enterovirus. The qPCR done for adenovirus was not specific for the gastroenteritis-causing serotypes 40 and 41. The detection of adenovirus in co-infections could, therefore, represent true gastroenteritis co-infections or shedding of any adenovirus serotype. In a previous study, non-enteric adenovirus serotypes represented 42% of detected adenoviruses in stool samples from children admitted to hospital with AGE.[34] Enterovirus has not classically been associated with AGE, but studies suggest that enterovirus may be a causative organism in AGE.[327-329] As with adenovirus, detecting enterovirus in a faecal specimen could represent a co-infection with more than one viral enteric pathogen or shedding of the enterovirus during an AGE episode with another pathogen.[330] Co-infections did not result in a more severe illness, likely because most single-pathogen infections were rotavirus

infections, and rotavirus often causes more severe infections than the other viral AGE pathogens.[38,42,43,312]

5.2.2 Disease burden of RVAGE

Rotavirus is a major causative organism in acute gastroenteritis in young children leading to utilisation of health care services,[32-35] as shown in our study with rotavirus in more than half of the samples with a detectable pathogen. RVAGE is most common among the youngest children, with 70% of children in our study ≤24 months of age, as also shown in other studies.[32,38,43,45,331] However, children with RVAGE were older than children with non-RV AGE (median of 18 months vs 14 months) in our study, as also reported elsewhere.[331-333] Protection by maternal antibodies in younger children might partly explain this age difference.

Rotavirus caused more severe illness than non-RV AGE; 80% of RVAGE were defined as severe based on the Vesikari score compared to 53% of the non-rotavirus AGE. Children with RVAGE received treatment in the ED more often, and more children with RVAGE than non-RV AGE were hospitalised. Similar findings have previously been reported.[38,312] This adds to the healthcare cost of rotavirus. Children with RVAGE presented sooner in the disease course to the ED than children with non-RV AGE, but the duration of illness was shorter for RVAGE than for non-RV AGE. Shorter hospitalisation for RVAGE compared to non-RV AGE has previously been reported.[331] That could be explained by the older age of children with RVAGE compared to children with non-RV AGE.

Rotavirus vaccinations have been shown to reduce the number of ED visits and hospital admissions due to RVAGE and reduce all-cause AGE hospitalisations.[35,41,62-64] The VE of the rotavirus vaccines has been estimated at ≥80% against laboratory confirmed RVAGE, ED visits and hospitalisations.[64,70,334] Importantly, Rotarix® and RotaTeq® have similar VE and are effective against both homo- and heterotypic strains.[71,334] Even in vaccine breakthrough infections, the infections have a shorter duration, are less severe, medical care is sought for fewer cases, and fewer children require intravenous rehydration.[64] The disease severity and clinical factors of breakthrough infections do not differ between the rotavirus vaccine brands.[64]

Rotavirus is highly contagious,[28] and RVAGE often leads to secondary cases within households.[40,312,333] In our study, around 60% of cases led to at least one secondary case in the household. Previous studies have reported 46-52% of cases leading to at least one secondary case in the home.[40,312]

Rotavirus has been reported to be more likely to cause secondary household cases than other gastrointestinal viruses,[312,333] but in our study, the relative number of secondary cases was similar between RVAGE and non-RV AGE. The high proportion of cases leading to secondary cases adds to the disease burden and increases the number of days lost from work. Furthermore, rotaviral illness affects the quality of life of children and their parents during the RVAGE episode. The disease causes suffering for the child, anxiety and distress for the parents and impacts daily life.[40,335,336]

In our study, the median number of days lost from work was four for each RVAGE episode, not accounting for secondary cases within the household. Other studies have reported a mean or median of 2-7.5 lost workdays.[40,64,88,312] Parental absence from work differs between studies and countries,[40,64,88,312] which may in part be attributed to different societal norms in terms of paid childcare,[88] duration of maternal/paternal leave and female participation in the job market.

RVAGE was most common in late winter and spring, a well-described seasonal pattern.[32,37,44,45] The peak of the rotavirus season hits at a similar time as influenza and respiratory syncytial virus (RSV),[46,47] which can cause a significant burden on healthcare facilities. Rotavirus vaccinations affect the seasonality by delaying the onset, moving the peak further into the spring and shortening the rotavirus season,[35,41,80,337] which can contribute substantially to reduced strain on healthcare facilities.

More cases of RVAGE were observed in 2018 than in 2017, both in absolute numbers and relative to the number of AGE cases. However, there were also more AGE cases diagnosed in 2018 than in 2017 and relatively better participation in the study. A biennial pattern in RVAGE was reported in the USA after the introduction of rotavirus vaccinations.[338,339] Other high-income countries that have included rotavirus vaccinations in the immunisation programmes have not observed the same pattern.[79,340,341] The biennial pattern is hypothesised to stem from low vaccine coverage, and it might be diminishing with increasing vaccine coverage in the USA.[342,343] Rotavirus vaccinations have not been included in the NIP in The Netherlands, but a biennial pattern of RVAGE has nevertheless been observed.[344] Herd immune effects from neighbouring countries with national rotavirus vaccinations were hypothesised to explain the biennial pattern observation in The Netherlands,[344] although a later study did not show evidence of that effect.[80] Whether the differences in RVAGE numbers between the two study years represent natural fluctuations in incidence or indicate a biennial pattern

cannot be determined with certainty, although the former is more likely. A more extended study period would have been needed to identify a pattern in RVAGE incidence.

5.2.3 Cost-effectiveness of rotavirus vaccinations

RVAGE causes substantial societal costs, both due to the costs of the healthcare system and due to the loss of productivity during the time parents take care of their sick children.[88,345]

In our study, we estimate that the annual societal cost of RVAGE is approximately €2.9 million (using costs and the official exchange rate of 2018), €2.5 million due to loss of productivity while parents care for their ill children and €369,677 in direct hospital costs. Loss of productivity has been reported to account for 36-73% of indirect societal costs due to RVAGE, with intercountry differences,[88] and is even higher in our study (86.2%).

Our estimate of societal costs due to RVAGE is rather cautious. We assumed a 10% annual incidence of RVAGE for children under the age of six. That is likely an underestimate since most children have had a rotavirus infection at least once by five years of age.[29,36] In our study of RVAGE leading to ED visits, the median number of missed workdays was four. To account for potentially fewer days missed from work for less severe infections that do not lead to ED visits, we assumed that each RVAGE would lead to three days of parental absence from work/school. Other studies have reported 2-7.5 lost workdays, so three days may be a conservative estimate.[40,64,88,312] We only included missed days from work due to the primary case, not secondary household cases, which would add even more missed days. When accounting for the cost of health care, we included the costs of ED visits and hospital admissions but not GP visits. Furthermore, direct costs that fall on families, such as costs due to increased diaper use and medications, were not considered. Also, no financial value was placed on parental worries, loss of free time or reduced quality of life due to RVAGE episodes. Despite the cautious estimate, our results suggest cost-effectiveness of rotavirus vaccinations in Iceland. Several studies have estimated the cost-effectiveness of rotavirus vaccinations in high-income countries and found rotavirus vaccinations to be cost-effective and even cost-saving.[65-69,88]

Rotavirus vaccinations could be added to the Icelandic NIP without additional infant care visits. The price of the vaccine is, therefore, the most significant determining factor regarding the addition of the vaccine to the NIP.

According to our analysis, the vaccine would be cost-saving given a 75% reduction in infection rate and €100 cost of each complete vaccination schedule.

5.2.4 Study strengths and limitations

The study has several strengths. The prospective design, with stool samples from participants, is more likely to paint the correct picture of the true rotavirus incidence than a retrospective study would have done, as stool samples are seldomly collected in clinical practice. qPCR was done for six enteropathogenic viruses, giving a comprehensive view of AGE of viral aetiology. Parents were contacted via telephone within one week from the ED visit to collect information about the total duration of symptoms and the number of days missed from work. The short time from the ED visit to the phone call limited the risk of recall bias. Contacting parents via telephone is likely to have contributed to limited missing data, as the follow-up data was not reliant on parents remembering to fill out forms or questionnaires. We included loss of productivity in the cost calculations, which accounts for a substantial amount of the societal costs.[88] Our study, therefore, gives a representative picture of the actual societal cost of RVAGE.

The study has some limitations. Only a third of children attending the ED with AGE symptoms were recruited for the study. Participation in the study depended on healthcare workers in the ED inviting parents to participate and on informed consent from parents/legal guardians. It is possible that disease severity influenced whether healthcare workers remembered to promote the study and the willingness of parents to participate, leading to a selection bias in the cohort. Furthermore, although vaccines are generally positively perceived in Iceland,[346] parental answers on their opinion on rotavirus vaccinations are likely biased since all parents had children that recently had an AGE episode.

The study period was two consecutive years, and there was a substantial difference in the number of AGE episodes between the two years. Some fluctuations in the identified causative pathogens were also noted. Using the average number of RVAGE cases during the two years, we believe we have a representative number for RVAGE cases.

Genotyping of rotavirus was not done, and it can, therefore, not be asserted that the vaccine genotypes are the prevailing rotavirus genotypes in Iceland. Substantial inter-country differences are in place regarding genotype

dominance in Europe.[347,348] However, both the monovalent Rotarix® and the pentavalent RotaTeq® have good VE against homo- and heterotypic strains.[71]

5.2.5 Should a rotavirus vaccine be included in the national immunisation programme in Iceland?

In Study I, we show that the disease burden of rotavirus on children, families, and the health care system is considerable. The costs of rotavirus infections are substantial, and rotavirus vaccinations would be cost-effective in Iceland. RVAGE impacts the quality of life of children and their parents and causes a significant burden on health care systems,[39-42,335,336] typically when the burden of respiratory infections is also high.[45-47] The rotavirus vaccines have been shown to greatly reduce the burden of RVAGE,[35,41,62-64] and WHO recommends the inclusion of rotavirus vaccinations in all national immunisation programmes.[49] More than 90% of parents of children participating in our study were in favour of including rotavirus vaccinations in the immunisation schedule. According to our analysis, rotavirus vaccinations are cost-effective in Iceland.[42] We conclude that rotavirus vaccinations should be added to the paediatric immunisation schedule in Iceland.

5.3 Study II - MENICE: Meningococcal colonisation in children, adolescents, and young adults in Iceland

Study II describes the meningococcal carriage prevalence among young children, adolescents, and young adults in Iceland. The longitudinal part of the study is, to our knowledge, the longest follow-up study on meningococcal carriage that has been conducted. We show that meningococcal colonisation can persist for at least 21 months. According to our study, prolonged carriage is likely due to the persistence of the same meningococcal strain rather than the reacquisition of a different strain.

5.3.1 Colonisation prevalence

The carriage prevalence in our study was 0% in children attending DCCs, 0.5% in adolescents and 6.5% in young adults, which was lower than expected. Most carriers were colonised with NG meningococci, as has also been reported by others.[113,114,122,140] Of the capsulated meningococci, MenB was most common, as has also been described in other European carriage studies.[122,124,140,349] None were colonised with MenC, the capsular group that is currently targeted in the NIP in Iceland.

No children attending DCCs were colonised with *Neisseria meningitidis*. Previous studies have reported 2-3% meningococcal colonisation prevalence

among young children.[117,350] In a meta-analysis of meningococcal carriage by age, the carriage prevalence was estimated to be 4.5% for infants, with a non-linear increase to 7.7% in 10-year-olds, reaching a peak of 23.7% in 19-year-olds.[118] Given a 2.5% colonisation prevalence, our study should have identified approximately 11 carriers in the youngest group.

Carriage of *Neisseria lactamica* is considered to protect against colonisation with *Neisseria meningitidis,* and the carriage of *N. lactamica* is highest in young children.[117,351,352] Competition between the two *Neisseria* species has been suggested as an explanation for the protective effects of *N. lactamica* colonisation.[126,353] *N. lactamica* colonisation has furthermore been shown to induce cross-reactive immune responses to *N. meningitidis.*[353,354] In inoculation studies of *N. lactamica*, the inoculation led to displacement of meningococcal colonisation and reduced acquisition rates,[126,353] although replacement of *N. lactamica* colonisation with *N. meningitidis* colonisation and subsequent IMD has also been described.[355] We did not look for *N. lactamica*, but high carriage rates of *N. lactamica* could be a part of the reason for the non-detectable carriage of *N. meningitidis* in the youngest age group in our study. Furthermore, 9.6% of participating children were reported by their parents/guardians to have been treated with antibiotics in the 30 days before the sample collection. High rates of antibiotic use in young children in Iceland likely contributes to the non-detectable meningococcal carriage in this age group.[356]

Colonisation prevalence was 0.5% in the adolescent group (15–16-year-olds), with only one adolescent carrying meningococci, which were non-groupable. In a study from the UK, conducted in 2014-2015, 5.9% of 15-16-year-olds carried meningococci.[132] The substantially lower carriage in our study might be explained by lower overall meningococcal colonisation prevalence in society, different circulating strains and differences in social norms.

Of the young adults, 6.5% carried meningococci. The carriage of non-groupable meningococci in the age group was 4.8%, and 1.7% for groupable meningococci (1.1% for MenB, 0.4% for MenY and 0.2% for MenW). The carriage was lower than anticipated in our study and lower than commonly reported carriage rates in cohorts vaccinated against MenC.[113,120,122,124,126] Similar colonisation prevalence (4.32-7.23%) as observed in our study has been reported in recent studies, which might be explained by changes in the social behaviour of adolescents and young adults.[132,133,146]

Non-groupable strains were the most common in asymptomatic carriage in Icelandic adolescents and young adults. NGs are usually non-pathogenic but have been reported to cause IMD.[102,103] Meningococcal isolates from IMD that are defined as non-groupable with slide agglutination can usually be genogrouped with molecular methods, but capsule null locus (*cnl*) isolates can also cause IMD.[102,171,357,358] Furthermore, non-groupable carriage strains can acquire capsule virulence genes resulting in increased virulence, as shown by the emergence of the ST-10217 outbreak strain in the African meningitis belt.[359]

5.3.2 Persistent carriage

In the longitudinal arm of this study, the follow-up was 27 months. That is, to our knowledge, the longest follow-up study on meningococcal carriage that has been conducted. The study shows that asymptomatic meningococcal colonisation can persist for at least 21 months (from the first positive to the last positive sample). The longest duration of colonisation with genogroupable meningococci was 12 months, with MenB. Both individuals carrying MenY had negative follow-up swabs after three months, and the one MenW carrier was lost to follow-up. Varying duration of carriage has been observed in previous longitudinal studies.[123,124,130,138,140] In our study, all persistent carriers had the same genogroup in every sample collected for the duration of their carriage. In other longitudinal studies, clonal replacement or capsular switching has been observed.[105,138,140,360] A recent longitudinal carriage study from Sweden showed that 62.5% of carriers with >1 positive sample carried the same strain in successive samples.[140]

To assess whether the abundance of *N. meningitidis* in the sample was associated with persistent carriage, 16S qPCR was done. We did not find higher absolute nor relative abundances of *N. meningitidis* in the first samples from prolonged carriers compared to non-prolonged carriers. However, the analysis only included three non-prolonged carriers when those lost to follow-up had been excluded, limiting the power of the comparison. On the other hand, both absolute and relative abundances of *N. meningitidis* were higher in 43 follow-up samples than in 35 baseline samples, indicating a higher abundance of meningococci in persistent carriage. Increased density in the first sample has previously been associated with increased odds of persistent carriage.[130]

5.3.3 Whole genome sequencing

All viable strains that were groupable by qPCR (*ctrA* and *metA* positive) were sequenced, a total of 14. The genogroup identified by qPCR was confirmed for ten strains, but four strains that were MenB according to qPCR were defined by WGS as non-groupable with a B backbone. Three of those had phase variable off in *csb,* a capsule biosynthesis gene for group B,[98,317] and in the fourth, *csb* was disrupted by an insertion element. Interestingly, strains with genogroup B were identified in three successive samples from carrier 0254. However, the strain in the fourth sample was non-groupable with a B backbone, with a phase variable off in *csb*. No other persistent carriers carried strains that changed genogroups during the study. In a previous longitudinal study, strains from a few persistent carriers had variable capsule expression over the follow-up period.[105] Genes encoding for various meningococcal surface antigens have been shown to have a tendency towards lower expression during persistent carriage,[361] likely in part due to a selective pressure mediated by antibodies against the colonising meningococci.[361] The phase variation has been hypothesised to facilitate the persistence of colonisation.[361] It is, therefore, fascinating that the fifth sample from participant 0254 was negative for meningococci. Additionally, non-groupable strains with a phase variable off in *csb* were identified in two successive samples from participant 0081, but the third sample was negative. The duration of carriage of non-groupable strains with phase variable off in *csb* in carrier 0081 before enrolment to the study is unknown. The numbers of strains with phase variable off in our study are too low to draw conclusions from, but our limited data does not suggest increased persistence with the *csb* phase variable off.

Whole genome sequencing of the capsulated strains in our study revealed a close relatedness between successive strains from the same participant, with 0-3 allelic differences between them. That suggests persistent carriage with the same strain.

CC-213 was the most common CC identified among capsulated strains in our study (9/14 sequenced strains, 64.3%). Six strains with CC-213 belonged to genogroup B, and three were non-groupable with a B backbone (phase variable off in *csb*). Eight of ten strains from prolonged carriers belonged to CC-213, and one from a non-prolonged carrier. The prevalence of this CC in persistent carriage might be explained by a better ability to maintain colonisation than other CCs or by it being the dominant colonising CC in adolescents and young adults in Iceland. Both MenY strains belonged to CC-

23, the predominant MenY CC in carriage and IMD, which is often reported as one of the most common CCs in asymptomatic carriage.[132,133,140] CC-32, CC-41/44 and CC-213 have also been reported to be common in meningococcal carriage.[133,140,146] All clonal complexes identified from carriers in our study (CC-213, CC-41/44, CC-23 and CC-32) belong to CCs that are among the most common in IMD.[151,362-364] That is interesting in light of the low incidence of IMD in Iceland and highlights that the progression from colonisation to IMD depends on various factors.

The MenDeVAR index on PubMLST and the WGS data were used to assess the reactivity of the two protein-based meningococcal B vaccines against the sequenced strains. All strains with CC-213 had an exact match to a Trumenba® vaccine antigen. In contrast, Bexsero® had no reactivity to seven of the nine CC-213 strains. Data was insufficient to conclude about Bexsero® reactivity for the other two CC-213 strains. Trumenba® was additionally determined to be cross-reactive against both CC-41/44 MenB strains, as well as both CC-23 MenY strains. A low reactivity of Bexsero® against CC-213 strains has been described in both the UK and the Netherlands, with only 10-23% of CC-213 MenB strains being covered by Bexsero®.[364,365] Generalisability of vaccine coverage of clonal complexes is difficult, though, due to the dynamic nature of antigen expression.[365]

5.3.4 Meningococcal vaccinations and antibiotic use

In total, eighty per cent of the adolescents and young adults were vaccinated against meningococci. The proportion of vaccinated participants differed between the two groups, with 89% of adolescents vaccinated compared to 78% of young adults. Furthermore, most adolescents had received two doses of a monovalent MenC vaccine, whereas almost all vaccinated young adults had received one dose. Monovalent MenC vaccinations were introduced in Iceland in 2002, with the addition of meningococcal vaccinations in the national childhood immunisation programme in a two-dose schedule at six and eight months of age and with a catch-up campaign for $\leq$19 year-olds.[184] Most individuals in the young adult group were born in 1999-2001 and were therefore offered the vaccination as a part of the catch-up campaign, which included one dose of a MenC vaccine. The adolescent group, however, born in 2003, received their vaccines as a part of routine childhood vaccinations. Possibly, the catch-up campaign did not reach all who should have been vaccinated. Suboptimal registration of the catch-up vaccinations compared to routine vaccinations may also contribute to an underestimation of the vaccine coverage in this age group. Furthermore, the Vaccination Register only dates

back to 2002/2003, so incomplete registration could also explain the lower-than-expected MenC vaccine coverage in the cohort, especially among young adults.

Monovalent MenC vaccinations have been shown to significantly reduce the carriage of MenC.[209-211] That is corroborated in our study, as we did not identify a single MenC carrier in the cohort. The same findings have been observed in studies from the UK and Portugal, with no MenC carriers in cohorts vaccinated against MenC.[120,121]

Five per cent of adolescent and young adult participants had filled prescriptions for antibiotics in the 30 days prior to the first swab (6.1% of adolescents and 4.6% of young adults), which may contribute to the low carriage rates. Some studies report higher carriage rates in cohorts with similar or higher antibiotic consumption, but the classes of antibiotics used are not reported.[119,120,140] It is possible that different conventions in the choices of antibiotics between countries play a part.

5.3.5 Tonsillectomies

In our study, previous tonsillectomies were associated with a three-fold increased likelihood of carrying meningococci. The same association has been reported in other studies.[140,366,367] One showed that a quarter of patients had become colonised with meningococci three months after a tonsillectomy compared to none of the controls.[367]

The reasons for the increased risk of meningococcal colonisation following tonsillectomies remain unclear, but immunological effects and changes in the microbiological flora might play a role. The tonsillar tissue is a part of the local immune system, but the immunological consequences of tonsillectomies have been debated, although most studies show little or no immunological consequences.[368-370] One study reported that tonsillectomies did not affect the serum levels of IgG, IgM, nor IgA in children in the year following tonsillectomy.[368] In another study, serum IgA and serum IgG1 decreased more in one year in 4-8-year-old children that had tonsillectomy, compared to a watchful waiting group.[369] Importantly, the incidence of throat and upper respiratory tract infections was not increased in the tonsillectomised group compared to the control group.[369] It is, therefore, unlikely that poorer immune responses explain the increased risk of meningococcal carriage associated with tonsillectomies.

The microbiological flora changes following tonsillectomies, with reduced carriage of potentially pathogenic bacteria, such as Group A streptococci,

Streptococcus pneumoniae and *Haemophilus influenzae.*[371,372] The microbiological ecosystem is complex, with interactions and competition between species affecting which bacteria maintain colonisation.[126,353,373] Removal of the tonsillar tissue might affect the pharyngeal microbiological flora and create a niche leading to an increased risk of meningococcal colonisation.

Meningococcal sepsis and meningitis following tonsillectomies have been reported,[374-376] but whether tonsillectomies increase the risk of IMD in the long term remains to be studied. Given the increased colonisation prevalence of meningococci following tonsillectomies and that colonisation precedes IMD, tonsillectomies might slightly increase IMD risk. Further studies are needed to shed light on the impact of tonsillectomies on meningococcal colonisation and their role in the development of invasive meningococcal disease.

5.3.6 Methodology

The choice of sampling site could affect the detection of meningococci. In our study, nasopharyngeal samples were collected from children attending DCCs but oropharyngeal samples from adolescents and young adults. Few studies address the optimal sampling site for the detection of meningococcal carriage, but studies suggest that oropharyngeal swabs are superior to nasopharyngeal swabs,[377,378] although some conflicting results exist.[121] The use of nasopharyngeal swabs in the DCC children may have contributed to the non-detectable carriage in that age group.

We placed swabs in a transport medium and plated them within six hours of collection. Direct plating has been reported to give a higher yield than the use of a transport medium.[118,379] It is conceivable that the carriage prevalence is underestimated due to the use of transport medium.

Conventional culture methods and MALDI-TOF MS were used to detect *N. meningitidis*, followed by qPCR on the culture-positive samples (both on OPSs and viable cultured strains). The MALDI-TOF MS lacks specificity regarding *Neisseria spp.* and tends to incorrectly identify commensal *Neisseria spp.* as *N. meningitidis.*[380-382] In our study, two culture and MALDI-TOF MS positive samples were negative on the qPCR of the cultured strain. Both had MALD-TOF MS scores <2, but according to the manufacturer, MALDI-TOF MS scores >2.0 should give a species identification with high confidence but scores ranging from 1.70-1.99 a low confidence identification.[383] However, 13% (9/69) of strains confirmed as *N. meningitidis*

by qPCR on a cultured strain had MALDI-TOF MS scores <2.0. This suggests that the MALDI-TOF MS scores do not provide certainty on the reliability of identification.

Due to the MALDI-TOF's lack of specificity, the use of additional tests to verify the results has been proposed.[381] This method has been used at the Department of Clinical Microbiology at Landspitali University Hospital. We tested 34 strains with the biochemical test API NH® to assess its ability to confirm MALDI-TOF MS identification of meningococci, using qPCR of cultured strains as a reference. API NH® identified two meningococcal strains as commensal *Neisseria spp.*, gave inconclusive results for one non-meningococcal strain, and correctly identified the other non-meningococcal strain as a commensal *Neisseria sp.* These findings suggest that using API NH® to confirm a MALDI-TOF identification of *N. meningitidis* is unreliable.

qPCR was done on both OPSs and cultured strains (except for the nine strains that were non-viable after freezing). qPCR on cultured strains detected additional eight positive samples that were qPCR negative on the OPSs, indicating a higher sensitivity of qPCR on cultured strains than OPSs. Additionally, qPCR was done on 73 culture-negative OPSs, of which one (1.4%) was *metA* positive. If 1.4% of culture-negative samples from the first sampling of adolescents and young adults were actually positive, that amounts to ten additional positive samples, giving a carriage prevalence of 6.2%, instead of 4.8%. This highlights that a combination of culture and molecular methods is helpful for detecting *N. meningitidis*.

For qPCR, the meningococcal-specific genes *metA* and *ctrA* were used as targets. *metA* has been shown to be very sensitive and specific for detecting *N. meningitidis*.[314,384] *ctrA* is a capsular transport gene and, therefore, absent in many non-groupable strains.[99,317] SNPs have also been reported in the primer and probe sequences of *ctrA*, which can cause false negative results on qPCRs targeting *ctrA*.[314] The use of *metA* in conjunction with either *ctrA* or *tauE* has been recommended,[314] as was done in our study. Using two gene targets greatly reduces the likelihood of false negatives.

5.3.7 Study strengths and limitations

Several limitations of the study must be addressed. First, only carriers at the first sampling time point were invited to participate in the longitudinal arm of the study, and the follow-up ended with the first negative swab. Therefore, we have no means of assessing intermittent carriage or the acquisition rate of meningococci. It has been argued that acquisition rates are of more

importance than carriage rates when determining the transmission of meningococci.[385] High carriage rates can be associated with a very low incidence of IMD. Low carriage rates but a highly transmissible strain and high acquisition rates can also be associated with high incidence of IMD.[117,146,385] Second, no social information was collected about the participants. Various social factors, such as smoking, attending pubs/clubs and kissing, have repeatedly been established as risk factors for meningococcal colonisation.[114,119,121,124,125,128,129] We did not collect information on current or recent upper respiratory tract infections, also risk factors for meningococcal carriage.[114,128] Third, there was some loss to follow-up between sampling time points. Fourth, not all samples were tested with qPCR, but in a selection of culture-negative swabs, 1.4% were qPCR-positive. It is, therefore, possible that the carriage prevalence is underestimated. Fifth, only the groupable strains were sequenced, so we do not have information about the relatedness of non-groupable strains from the same carrier and cannot infer that it is, in fact, the same non-groupable strain that maintains colonisation for several months.

The study has many strengths. No other study that we know of has followed meningococcal carriers for this long. Molecular methods were used to determine the meningococcal genogroups and WGS was utilised to assess the relatedness of meningococcal strains during prolonged colonisation. Information on vaccinations and antibiotic use was acquired on an individual level, allowing for a more detailed assessment of the effects of meningococcal vaccinations and antibiotic use on meningococcal carriage. In addition, the information about antibiotic use, vaccinations and tonsillectomies was collected from central databases governed by the Directorate of Health Iceland and the Icelandic Health Insurance as well as from Landspitali University Hospital, rather than from the participants themselves, eliminating the risk of recall bias.

5.3.8 Should changes in meningococcal vaccinations be made to the Icelandic national immunisation programme?

The prevalence of meningococcal colonisation in our study was low, with only 0.5% of adolescents and 6.5% of young adults carrying meningococci. When only capsulated meningococci are considered, the carriage prevalence among young adults was 1.1% for MenB, 0.4% for MenY and 0.2% for MenW. No participant carried MenC or MenA and no adolescent carried capsulated meningococci. Considering also the very low incidence of IMD in

Iceland, our results do not suggest a need for an altered meningococcal immunisation programme in Iceland.

A quadrivalent MenACWY vaccine was introduced in the NIP for toddlers in the summer of 2023 since the monovalent MenC vaccine was no longer available. Changing from a MenC to a MenACWY vaccine will provide increased coverage in protection, including capsular groups that have been increasingly common in some European countries for the past years. On the other hand, studies show conflicting results regarding the effects of MenACWY vaccinations on meningococcal carriage,[131,146,210] whereas the monovalent MenC vaccine is also protective against carriage.[209,210] The change could, therefore, potentially affect herd immunity.

The need to add a MenB vaccine to the NIP is debatable as not much carriage was detected in our study, and very few MenB IMD cases have occurred in the past few years. Adding MenB vaccinations to the NIP would provide protection against the most common capsular group in both colonisation and IMD in Iceland and Europe. Furthermore, the MenB vaccines provide cross-protection against other capsular groups.[201-205] However, cost-effectiveness analyses in high-income countries have deemed MenB vaccinations as unlikely to be cost-effective due to high vaccine prices and low incidence of MenB IMD.[386-388] MenB vaccinations were, however, estimated to be cost-effective in the UK (given the incidence of 1.83 infections per 100,000 people), when the broad burden of disease, including long-term sequelae, burden on family members and cost of outbreak management, was taken into account.[389] The ethical considerations of the availability of an effective vaccine against a very serious, albeit not common, disease and not including it in the NIP can also be debated.

When pentavalent MenABCWY vaccines will be licenced, the feasibility of changing from a quadrivalent MenACWY to a pentavalent MenABCWY vaccine to provide protection against all the major capsular groups should be considered. Continued thorough surveillance is important, with swift responses if needed, with a potential addition of MenB vaccines to the NIP or booster doses of MenACWY in adolescence.

5.4 Study III - OFICE: Influenza vaccine responses in adolescents with obesity

In study III, we found that the early humoral and cellular immune responses to tetravalent influenza vaccinations are similar between adolescents with obesity and adolescents with normal weight. Most participants in both groups

seroconverted after vaccinations, and a similar proportion of T cells secreted the studied cytokines in response to stimulation with the vaccine influenza strains.

5.4.1 Humoral immune response

In our study, the pre-vaccination titres were similar between the two groups, as reported in a previous study.[283] Seroprotective titres (≥40) were already present pre-vaccination in more than half of the participants in our study against influenza A/H1N1, A/H3N2 and B/Yamagata. The proportions of participants with pre-vaccination seroprotective titres in other studies have ranged from 13% to 93%.[282,285] Seroprotection against the B/Victoria strain was less prevalent, with 27% of the participants in the study group and 17% in the control group having seroprotective titres. The high prevalence of seroprotective titres pre-vaccination is likely explained by a cross-protective immune response to previous influenza infections.

A similar increase in antibody titres occurred following vaccination in both groups, although the study group had higher post-vaccination titres against influenza B/Victoria. Post-vaccination antibody titres have previously been shown to be similar in children, adolescents and adults with and without obesity.[279,283,285] However, increasing BMI in adults has been associated with a larger reduction in the antibody titres 12 months following vaccination.[279] In a study of children and adolescents, the antibody titres remained high in the group with obesity six months after vaccination and were similar to the titres in the non-obese group.[285] That is reassuring and suggests that the protection from the vaccination lasts throughout the influenza season.

The majority of participants had seroprotective titres post-vaccination, as has also been reported in another study.[285] One previous study reported that seroprotective titres were more often reached against influenza A/H1N1 in children with overweight or obesity than children with normal weight a month after the vaccination.[282] The reasons for the different results between studies are unknown. The study and the control groups had similar seroconversion rates in our study, ranging from 80% against influenza B/Yamagata in the control group to 100% against influenza A/H1N1 in the study group and influenza B/Victoria in the control group. Increased seroconversion but not seroprotection against influenza A/H3N2 has been reported in older adults with obesity compared to normal-weight older adults, but neither increased seroprotection nor seroconversion against influenza A/H1N1.[280] According to our results, obesity in adolescence does not seem to affect seroconversion or seroprotection rates.

The male participants in the control group had higher post-vaccination titres against influenza A/H3N2 than females in the control group. The difference did not remain significant after correcting for multiple testing and is most likely due to chance. In general, immune responses to vaccinations and infections are stronger in adult females than males.[390] Post-vaccination titres were higher against both influenza A/H3N2 and B/Victoria in females in the study group compared to females in the control group. The reasons for this are unknown, but it could be due to chance, as the difference was not significant after correcting for multiple testing.

Projecting laboratory data to clinical protection is challenging. Traditionally, a GMT of at least 40 is used as a definition of a seroprotective titre, indicating 50% protection against clinical infection.[391,392] The seroprotective titre may vary with age, as a study on 6-72-month-old children estimated a titre of 85 to give 50% protection and a titre of 302 to give 80% protection.[393] It is unclear which GMT value corresponds to 50% protection in adolescents, and it may even vary with age within the adolescent group. Furthermore, obesity might also influence the protection of vaccination. In one study, influenza and ILI were more common in vaccinated adults with obesity than in vaccinated adults with normal weight, although similar proportions of infected individuals in both groups reached seroconversion and seroprotective titres.[394] Further studies are needed to determine whether adolescents with obesity are at increased risk for influenza infection compared to adolescents with normal weight and to ascertain the antibody titres corresponding to clinical protection.

5.4.2 Cellular immune response

The T cell responses to stimulation with the influenza vaccine strains, both for $CD4^+$ and $CD8^+$ cells, were similar for the two groups. The only difference was a higher proportion of pre-vaccination $CD4^+$ cells secreting TNF-α in response to stimulation with influenza A/H3N2 in the study group compared to the control group. After correcting for multiple comparisons, the difference was not significant.

Reduced activation and function have been reported in $CD4^+$ and $CD8^+$ T cells from adults with overweight and obesity compared to cells from adults with normal weight when stimulated with influenza.[281] The cells from individuals with overweight and obesity had diminished expression of the activation marker CD69, and the activated cells had reduced expression of CD28, CD40 ligand, IFN-γ, granzyme B and IL-12 receptor.[281] Interestingly, interleukin five (IL-5) levels were higher in people with obesity than people

with normal weight, which suggests the development of a Th2 response instead of a Th1 response.[281] Furthermore, proportionally less increase in $CD8^+$ T cells expressing CD69 and in activated $CD8^+$ T cells that express granzyme B and IFN-γ in PBMCs have been reported from adults with obesity compared to PBMCs from adults with normal weight 12 months after influenza vaccination.[279]

Also, higher circulating levels of IL-6 have been reported in children with overweight and obesity compared to peers with normal weight.[278] This is believed to reflect a chronic, low-level inflammation in individuals with overweight and obesity, which could affect the immune response. Interestingly, the same study did not show any difference in the circulating levels of TNF-α, IL-1β and IL-1ra between the children with obesity/overweight and the children with normal weight.[278]

There was no difference in the $CD4^+/CD8^+$ ratio between the two groups in our study, neither pre- nor post-vaccination. An elevated $CD4^+/CD8^+$ ratio has been shown in adults with class III obesity compared to adults with normal weight.[395] The discrepancy between the two studies could be explained by both differences in age and the composition of the study groups.

The results from study III do not suggest reduced cellular immune responses in adolescents with obesity compared to adolescents with normal weight, as has been reported in adults.[279,281] However, only four cytokines were measured, and there may be a difference in other markers or cytokines suggesting differences in responses between the two groups. Moreover, the study did not include a long-term follow-up of the cellular immune response.

5.4.3 Study strengths and limitations

The study has some limitations. First, the humoral immune response was evaluated with a HAI assay only. Microneutralization (MN) assay was not done, which may be more sensitive than the HAI assay.[262] Studies have, however, shown a strong correlation between HAI and MN assays.[262,396,397] Since almost all participants in our study had post-vaccination seroprotective titres and most seroconverted, it is unlikely that an MN assay would have altered the results of the study. Furthermore, similar studies also use HAI assay to assess the humoral response. Using the same method allows for a better comparison between studies.[282-285]

Second, the number of participants needed in the study to indicate a 25% difference in GMTs was determined with power calculations, but power

calculations were not done to assert how many participants would be needed to show a difference in the T cell stimulation assay.

Third, the relatively low numbers of live cells in the samples affected the ability to stimulate all samples with the four vaccine strains to measure the secretion of the four studied cytokines in the T cell stimulation assay. Also, IFN-γ analysis was not possible on 30 samples because of a technical error.

Fourth, the study only included an analysis of the early immune response to influenza vaccination; we did not include a long-term evaluation of the immune response. Evidence exists that the duration of the immune response might be shorter in individuals with obesity. Therefore, the study group may have had a faster waning immune response than the control group.

Fifth, we did not collect any information or do any measurements to assess the metabolic and hormonal profile of the participants. Participants likely have diverse metabolic profiles, which might affect the results. However, a recent study showed similar humoral immune responses in children with normal weight, metabolically healthy children with overweight/obesity and metabolically unhealthy children with overweight/obesity. The only difference observed between the groups was lower IL-13 concentrations in the group of metabolically unhealthy children with overweight/obesity, compared to the other groups.[284]

Sixth, the number of male and female participants were not equal, with 43% of study group participants being female and 30% of control group participants (not a statistically significant difference between the two groups). Previous studies have shown higher antibody titres in adult and elderly females than males.[279,398] If the same pattern holds true for adolescents, we might have underestimated the antibody titres in both the study and the control group.

Seventh, six participants in the control group were not normal weight, as assumed at recruitment. Four were underweight, and two were overweight. When the data was analysed excluding non-normal weight control group participants, the differences in GMTs against the B/Victoria strain and pre-vaccination $CD4^+$ TNF-α secretion between the two groups did not remain significant. Other results were not affected.

Our study has numerous strengths. First, the study participation was excellent. Only one participant from the study group was unable to complete the study participation, resulting in study completion for 98% of participants. Second, the study group was comprised only of adolescents with obesity, not

a combination of adolescents with overweight and obesity. Most previous studies addressing the same topic have included study groups with children and adolescents with overweight and obesity.[282-284] By only including adolescents with obesity in our study, we can more confidently assert that obesity does not influence the early immune response to influenza vaccinations. Third, we assessed both the humoral and the cellular immune responses, giving a more comprehensive evaluation of the immune response to the vaccination.

5.4.4 Are changes in influenza immunisations needed for adolescents with obesity?

In study III, we show that the early immune responses to tetravalent influenza vaccinations are similar in adolescents with obesity and adolescents with normal weight. Our results do not suggest the need for altered immunisations for adolescents with obesity, such as increased doses or longer needles. One study has shown similar humoral responses in children with obesity and children with normal weight six months after vaccination,[285] but further studies are needed to assess the longevity of the cellular immune response. Children and adolescents with obesity are not routinely vaccinated against seasonal influenza in Iceland. Annual influenza vaccinations should be considered for children and adolescents with obesity, as they may be at increased risk of severe influenza infections, and our study does suggest protective effects from the vaccination.

5.5 Study IV - FluRisk: Influenza vaccine uptake and burden of influenza illness in pregnant women and their infants

In study IV, we show that influenza vaccine uptake in pregnant women in Iceland was suboptimal in the influenza seasons 2010-2020. The vaccine uptake increased over the study period, from 6.2% in 2011-2012 to 37.5% in 2019-2020. Influenza vaccinations in pregnancy are protective against influenza/ILI in pregnant women and their infants in the season of vaccination and provide probable protection for infants <6 months of age.

5.5.1 Influenza vaccine uptake in pregnant women

The overall vaccine uptake over the ten influenza seasons was 19.1%, ranging from 6.2% in 2011-2012 to 37.5% in 2019-2020. Influenza vaccine uptake in pregnant women has been reported to be suboptimal in many other studies, with 7.5% to 45% of pregnant women vaccinated against influenza.[295,302,307,308,399-401] The low influenza vaccine uptake (7.3%) in our study in the influenza season 2010-2011 is surprising, considering the

influenza pandemic in 2009.[402] An increased awareness of the potential severity of influenza in pregnancy and the importance of vaccinations would have been expected following a pandemic.

There was a trend of increased vaccine coverage over the ten-season study period. The emergence of COVID-19 at the end of 2019 is unlikely to be the reason for the substantial increase in influenza vaccine uptake in the season 2019-2020, as 79% of the pregnant women vaccinated in that season were vaccinated in September, October or November, prior to the onset of the COVID-19 pandemic.[403,404] Other factors, such as increased recommendations by health care providers or awareness by pregnant women, are more likely to explain the increased vaccine uptake. It is, however, plausible that COVID-19 has led to increased awareness about influenza vaccinations and increased vaccine uptake since, but the past three influenza seasons (2020-2021, 2021-2022 and 2022-2023) were not included in the current study.

The determinants of influenza vaccine uptake in pregnancy have been the subject of several studies.[308,399,401,405,406] Recommendations from healthcare providers and good knowledge of influenza and influenza vaccinations are among the determining factors for acceptance of influenza vaccinations.[399,401,406] Along the same lines, reasons for not accepting influenza vaccinations have been reported to be a lack of knowledge about the vaccine and concerns about safety or adverse effects for the unborn child or the mother.[399,401,405] This emphasises the importance of healthcare providers being well informed about the safety and benefits of influenza vaccinations in pregnancy so that pregnant women are offered the vaccine and discussions about potential hesitations can be had.

The current study did not include questionnaires about pregnant women's attitudes towards influenza vaccinations in pregnancy nor about how well-informed healthcare providers are about the safety of influenza vaccinations in pregnancy, their attitudes towards influenza vaccinations and how comfortable they are discussing vaccinations with pregnant women. The attitudes towards influenza vaccinations and their benefits have been studied among staff at Landspitali University Hospital. In general, healthcare workers had positive attitudes towards influenza vaccinations and believed them to be beneficial.[407] It is, therefore, likely that other factors explain the low vaccine coverage, such as lack of recommendations from health care providers and inadequate patient education. To our knowledge, no studies have been done to assess the determinants of influenza vaccine uptake in pregnant women in

Iceland, and further studies are needed to shed light on the barriers to maternal influenza vaccinations in Iceland.

5.5.2 Disease burden of influenza in pregnant women

The incidence rate of influenza among pregnant women ranged from 5.5/1,000 person-years in 2010-2011 to 22.1/1,000 person-years in 2015-2016. Our results suggest that influenza vaccinations in pregnancy provide protection against influenza/ILI in the season of vaccination, with an estimated overall protection of 64% for the ten influenza seasons. The VE in individual seasons ranged from 34% to 100%. Similar results have previously been reported, with 44-50% VE against influenza infections and 40% against influenza-associated hospitalisations.[291,293,294] In our study cohort, seven pregnant women had influenza-associated hospitalisations, none of whom were vaccinated. That suggests vaccine protection against hospitalisations, although the numbers are too small to draw conclusions from.

Our study included influenza/ILI diagnoses in the influenza season the woman attended a 20-week ultrasound, the influenza season that is most likely to overlap with the largest proportion of the pregnancy. Influenza vaccinations in pregnancy have been reported to provide some cross-protection against influenza infections in the influenza season following the vaccination.[408] In addition to protecting the vaccinated woman, it may reduce the risk of influenza transmission to the infant, even if the mother is not vaccinated again in the following season.

5.5.3 Disease burden of influenza in infants

No infants were registered for 1,046 pregnancies in the cohort. Additional information was not collected on those pregnancies, so the reasons are unknown. It is likely explained by a combination of miscarriages, women living abroad attending an ultrasound at Landspitali University Hospital and women giving birth in other countries.

We found maternal vaccinations to be protective against influenza/ILI in the season of maternal vaccination and probably protective for infants <6 months of age. No infants diagnosed with influenza/ILI born to vaccinated mothers were hospitalised with influenza/ILI in the season of vaccination nor their first six months of life, indicating probable protection against influenza-associated hospitalisations from maternal vaccinations. In previous studies, influenza vaccinations in pregnancy have been shown to protect infants <6 months of age against influenza and reduce the risk of influenza-associated hospitalisations.[291,299,301-303]

The incidence rate for infants born to vaccinated mothers was highest in the season 2016-2017, both in the analysis of influenza/ILI in infants in the season of maternal vaccination and infants <6 months of age. In one study, maternal influenza vaccinations were not found to be protective against influenza B strains,[299] but influenza A/H3N2 was the predominant strain in 2016-2017 (unpublished data), indicating another reason for the high incidence rate in the season. The influenza vaccine effectiveness differs by season, and it may have been generally low in 2016-2017. That is supported by the fact that the season 2016-2017 was the season with lowest estimated VE for pregnant women in our study. The point estimate for VE in individual seasons for infants <6 months of age in our study ranged from -79% in 2016-2017 to 100% in six different seasons. The number of influenza/ILI cases in each influenza season was small, thereby reducing the accuracy of the vaccine effectiveness estimates. The VE for infants under the age of six months has been estimated at 35% to 63% in other studies.[291,299,301]

In our study, 47.4% of vaccinated pregnant women were vaccinated in the second trimester, 21.1% in the third trimester and 20.9% in the first trimester. Additionally, 10.7% had been vaccinated before the estimated start of the pregnancy. We did not consider infants born to mothers vaccinated <14 days before delivery as protected by the mother's vaccination. The best timing for influenza vaccination in pregnancy to achieve maximum protection has not been established and it might be different for maternal and infant protection.[292,299] In one study, no difference in infant protection was observed based on the timing of maternal vaccination.[299] The results from our study suggest the same, although the number of influenza/ILI cases is small. A study measuring antibody titres after vaccinations in the second and third trimester, given more than 15 days before delivery, showed that the timing between 120 and 15 days prior to delivery may provide the highest rise in antibody titres.[409] In a meta-analysis, vaccinations administered in later trimesters were shown to result in higher antibody titres in cord blood, compared to vaccinations earlier in pregnancy, indicating higher antibody titres in the newborn.[410] However, the clinical relevance of the higher antibody titres is uncertain.[409,410]

Twelve per cent (21/175) of infants <12 months of age diagnosed with influenza/ILI were hospitalised. The median duration of hospitalisation was two days. Other studies have reported a mean or median duration of hospitalisation ranging from two days to 6.6 days.[244,300,411] Of infants <6 months of age diagnosed with influenza/ILI, a third was hospitalised, suggesting a significant disease burden from influenza in this young age

group. None of them were born to vaccinated mothers. Previous studies have shown that maternal vaccinations decrease the risk of influenza-associated hospitalisations in infants.[302,304]

Five of the 21 hospitalised infants were diagnosed with potential influenza complications: acute otitis media, pneumonia, bronchiolitis, laryngotracheitis, transient neonatal neutropenia and acute tubulointerstitial nephritis. However, no infants were so severely ill that they were admitted to NICU or ICU. In other studies, 3% of infants <6 months of age admitted to hospital with influenza were admitted to an ICU and 2-3% of children <2 years of age, respectively.[244,300] No influenza-related deaths were registered for infants in our study cohort over the ten influenza seasons. Studies from other countries have reported 0.5-1.4 deaths per 100,000 children under the age of 12 months from seasonal and/or pandemic influenza.[248,251,411]

Thirty per cent of the infants in the cohort diagnosed with influenza/ILI were prescribed antibiotics in the four weeks following the influenza/ILI diagnosis. That indicates a high burden of bacterial co- or secondary infections. Influenza-associated illness has previously been shown to lead to antibiotic prescriptions frequently.[241] Our results do not suggest that maternal influenza vaccinations reduce antibiotic prescriptions for infants diagnosed with influenza/ILI. The frequent antibiotic prescriptions might also be explained by a low threshold for prescribing antibiotics to young children rather than the true burden of bacterial co- or secondary infections.

Maternal influenza vaccinations were protective against influenza in the season of maternal vaccination and probably protective for infants <6 months of age. The median age at diagnosis was, however, 8.4 months. At that age, the protection from maternal vaccination has diminished.[299] No influenza vaccines are licenced for infants under the age of six months, but the addition of influenza vaccinations for infants and young children from six months of age should be considered to protect this vulnerable group.

5.5.4 Study strengths and limitations

The study's main strength is that data on influenza vaccinations and influenza/ILI diagnoses was collected from central national registers governed by the Directorate of Health. They include data on all ICD-10 diagnostic codes from the public health care system, all laboratory-confirmed influenza diagnoses, and all registered vaccinations. Using central, national registers allows for a comprehensive view of the influenza vaccine uptake and the disease burden nationally. Additionally, influenza/ILI diagnoses from

primary care, outpatient visits and hospitalisations were included in the study, thereby including a spectrum of disease severity.

The study has some limitations. First, the cohort of pregnant women included women that attended a 20-week ultrasound at Landspitali University Hospital. We may have missed some women attending a 20-week ultrasound at other health institutions. However, most women in Iceland attend the 20-week ultrasound at Landspitali, and we believe the study's results represent the influenza vaccine uptake in Iceland. Second, women attending a 20-week ultrasound in May, June and July were excluded from the study to limit the risk of underestimation of influenza vaccine uptake since those pregnancies are unlikely to have had antenatal appointments in the influenza vaccination period. However, we may have missed some vaccinations at the start of the influenza vaccination period by excluding women attending 20-week ultrasounds in the summer months. Third, some employers offer their employees influenza vaccinations, which may not be registered in the Vaccination Register. Therefore, the influenza vaccine uptake may be slightly higher than reported in the study. Fourth, no information was collected on maternal age, parity, underlying medical conditions, educational level, socioeconomic status or other factors that may impact the influenza vaccine uptake.[308,399,400,406] Information was not collected either about breastfeeding of infants, the gestational age at birth and other factors that could influence the infant's risk of influenza/ILI. Fifth, information was not collected on the influenza strains in laboratory-confirmed influenza infections, but the circulating influenza strains may explain the differences in disease burden and vaccine effectiveness by season.

5.5.5 Are improvements needed in influenza vaccinations for pregnant women?

Influenza vaccinations in pregnancy provide protection against influenza for pregnant women and their infants in the season of vaccination. They furthermore provide probable protection for infants <6 months of age. The influenza vaccine uptake was suboptimal in the ten influenza seasons included in the study, although it increased gradually from 6.2% in 2011-2012 to 37.5% in 2019-2020. Despite the increased uptake, there is still room for significant improvement. Education should be provided for pregnant women on the benefits and safety of the vaccine, both for the mother and her unborn child. Healthcare providers have an important role to play in recommending influenza vaccinations. Initiatives are needed to increase influenza vaccine uptake in pregnancy.

6 Conclusions

All aims of the studies presented in this thesis were met.

Rotavirus is the leading cause of acute gastroenteritis leading to emergency department visits in young children in Iceland. It causes a significant burden on children and healthcare systems. RVAGE is more severe than other common viral causes of AGE and more often requires treatment in the ED. RVAGE also places a significant burden on society, with parental days lost from work due to their children's illness and the costs associated with loss of productivity. Vaccinations against rotavirus are cost-effective in Iceland. Rotavirus vaccinations should be added to the Icelandic immunisation programme, as recommended by the World Health Organization. Post-implementation surveillance of RVAGE epidemiology will shed light on the benefits of the vaccinations.

Meningococcal carriage is relatively uncommon in Iceland, with 0% of young children, 0.5% of adolescents and 6.5% of young adults carrying meningococci. The colonisation prevalence of capsulated meningococci is very low, with only 1.7% of young adults carrying capsulated meningococci and none of the adolescents and young children. Meningococcal colonisation can persist for at least 21 months, and our results suggest that prolonged colonisation occurs with the same meningococcal strain rather than reacquisition of a different strain. Further analysis of WGS data from the study remains to be done. Sequencing of NGs was not done, but it would be interesting to sequence and study the within-host evolution of NG meningococci compared to the groupable meningococci. Furthermore, only those that had a positive first swab were included in the longitudinal arm of the study, and the follow-up ended with the first negative swab. Should a longitudinal carriage study be repeated, it would be interesting to include a larger group in the follow-up and not conclude the follow-up with the first negative swab to study acquisition and the possible reacquisition of meningococci.

Considering both the low carriage of vaccine-preventable meningococcal groups and the low incidence of IMD in Iceland, our study does not suggest the need for alterations to the NIP regarding meningococci. However, changes are being made to the NIP, as the monovalent MenC vaccine is being replaced by a quadrivalent MenACWY vaccine for young children in a

one-dose schedule at 12 months. Whether that affects the colonisation and herd protection in children, adolescents, and young adults in Iceland is a topic for future studies. Furthermore, colonisation prevalence is not a constant, so carriage studies with regular intervals are key to assessing changes in meningococcal carriage dynamics. Studies assessing carriage of various bacteria among children in DCCs in Iceland have been done every winter for the past years. Adding *N. meningitidis* and *N. lactamica* to the yearly surveillance would shed light on the colonisation dynamics of the two bacteria in this age group.

Adolescents with obesity have similar early humoral and cellular responses to influenza vaccinations as adolescents with normal weight. The results from our study do not suggest the need for altered methods in influenza vaccinations for adolescents with obesity, such as a change in dose or method of administration. However, further studies are needed on the longevity of the immune response in adolescents with obesity compared to adolescents with normal weight. More studies are also needed to assess how well the laboratory measurements correlate to clinical protection. Furthermore, whether adolescents with obesity are at risk for more severe influenza than adolescents with normal weight remains to be determined.

Influenza vaccine coverage among pregnant women was suboptimal in Iceland in the influenza seasons 2010-2020, although it increased over the ten seasons, reaching 37.5% in 2019-2020. Influenza vaccinations in pregnancy provide protection against influenza for pregnant women and their infants in the season of vaccination and probable protection for infants in their first six months of life. The next steps are assessing the barriers to influenza vaccinations among pregnant women and to advocate for better influenza vaccine coverage.

In conclusion, the following conclusions can be drawn from the studies presented in this thesis:

- Rotavirus causes a significant disease burden on young children in Iceland, and rotavirus vaccinations should be added to the national immunisation programme in Iceland.
- The colonisation prevalence with vaccine-preventable meningococcal capsular groups is low in Iceland. Considering the low carriage and low incidence of IMD, our data does not suggest the need for changes in meningococcal vaccination strategies in Iceland. However, continued thorough surveillance and quick responses are important.

- Adolescents with obesity have similar early immune responses to influenza vaccinations as adolescents with normal weight. Our results do not suggest the need for adjustments in influenza vaccinations for adolescents with obesity.
- Influenza vaccinations in pregnancy are protective for the women and their infants in the season of vaccination and provide probable protection for infants <6 months of age. Influenza vaccine uptake among pregnant women in Iceland was suboptimal in the seasons 2010-2020. Initiatives are needed to increase influenza vaccine uptake in pregnancy.

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Appendix

Informed consent form for the RICE study (in Icelandic)

LANDSPÍTALI
HÁSKÓLASJÚKRAHÚS

Rannsóknarnúmer (t.d. R021): R_ _ _

Samþykki fyrir þátttöku í rannsókninni

Bráðar garnasýkingar hjá börnum yngri en 6 ára

Á Barnaspítala Hringsins er yfirstandandi rannsóknin **Bráðar garnasýkingar hjá börnum yngri en 6 ára.**

Rannsóknin er unnin í samvinnu Barnaspítala Hringsins og Háskóla Íslands.

Í rannsókninni verða tekin hægðasýni hjá börnum sem hafa einkenni um bráða garnasýkingu (uppköst og/eða niðurgang). Ef barnið skilar ekki hægðum á meðan dvöl stendur á spítalanum verður tekið húðstrok af svæðinu í kringum endaþarminn. Sýnin verða notuð til greiningar á veirum og bakteríum sem geta valdið garnasýkingum.

Hjá þeim sem samþykkja að taka þátt verður einnig óskað eftir að foreldrar fylli inn stuttan spurningalista (5 spurningar) 7 dögum eftir að veikindin hófust. Allar upplýsingar eru geymdar í læstum gagnagrunni sem aðeins starfsfólk rannsóknarinnar hefur aðgang að.

Ég samþykki að ég/barn mitt taki þátt í rannsókninni: Bráðar garnasýkingar hjá börnum yngri en 6 ára. Ég hef fengið og kynnt mér upplýsingar fyrir þátttakendur varðandi rannsóknina. Ég hef einnig fengið tækifæri til að spyrja spurninga varðandi rannsóknina. Mér er kunnugt um að ég get dregið þátttöku barns míns/mína til baka hvenær sem er.

Dagur og staður : _____

Nafn barns og kennitala: _____

Nafn foreldris/forráðamanns og kennitala: _____

☐ **Ég heimila að saursýni til veiru- og bakteríugreiningar verði tekið af barninu mínu**

☐ **Ég heimila rannsakendum að hafa samband við mig til að svara 5 spurningum varðandi lengd veikinda og áhrif á fjölskylduna.**

☐ **Ég heimila rannsakendum að hafa samband við mig að nýju ef niðurstöður rannsóknarinnar leiða til nýrra spurning og leyfi vísindasiðanefndar fyrir frekari rannsókn liggur fyrir**

Nafn þess sem leggur samþykkisyfirlýsinguna fyrir:_____

Informed consent form for the MENICE study, for adolescent participants (in Icelandic)

Rannsóknarnúmer (t.d. M021): M_ _ _

Samþykki fyrir þátttöku í rannsókninni:

Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum

Í samvinnu Háskóla Íslands og Barnaspítala Hringsins er í gangi rannsóknin:

Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum

Í rannsókninni verða tekin nefkokssýni hjá börnum á leikskólaaldri annars vegar og ungmennum 15-20 ára hins vegar. Sýnin verða notuð til að greina bakteríur í nefkoki hjá þeim sem taka þátt. Einnig verður leitað að öðrum bakteríum sem jafnan eru í nefkoki barna og ungmenna.

Hjá þeim sem samþykkja að taka þátt verður einnig óskað eftir grunnupplýsingum s.s. kennitölu, kyn og aldur auk símanúmers og/eða tölvupósts og að kanna hvort barnið hafi fengið bólusetningu gegn meningókokkum. Búist er við að um 0-20% þátttakenda beri meningókokka og óskum við eftir leyfi til að hafa samband við þá einstaklinga til að taka aftur sýni í 3 skipti til viðbótar þar til bakterían finnst ekki lengur (hámark 3 skipti og hámark 6 mánuði). Allar upplýsingar eru geymdar í læstum gagnagrunni sem aðeins starfsólk rannsóknarinnar hefur aðgang að.

Ég samþykki að ég/barn mitt taki þátt í rannsókninni: Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum.

Ég hef fengið og kynnt mér upplýsingar fyrir þátttakendur varðandi rannsóknina. Mér er kunnugt um að ég get dregið þátttöku barns míns/mína til baka hvenær sem er.

Dagur og staður : _____

Nafn þátttakanda og kennitala: _____

Nafn foreldris/forráðamanns og kennitala: _____

☐ **Ég heimila að nefkokssýni verði tekið úr mér/barninu mínu**

☐ **Ég heimila rannsakendum að hafa samband við mig ef meningókokkar finnast til að taka ný sýni.**

☐ **Ég heimila rannsakendum að hafa samband við mig að nýju ef niðurstöður rannsóknarinnar leiða til nýrra spurninga og leyfi vísindasiðanefndar fyrir frekari rannsókn liggur fyrir**

Nafn þess sem leggur samþykkisyfirlýsinguna fyrir:_____

Informed consent form for the MENICE study, for participants ≥ 18 years old (in Icelandic)

UNIVERSITY OF ICELAND

Rannsóknarnúmer (t.d. M021): M_ _ _

Samþykki fyrir þátttöku í rannsókninni:

Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum

Í samvinnu Háskóla Íslands og Barnaspítala Hringsins er í gangi rannsóknin:

Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum

Í rannsókninni verða tekin nefkokssýni hjá börnum á leikskóla aldri annars vegar og ungmennum 15-20 ára hins vegar. Sýnin verða notuð til að greina bakteríur í nefkoki hjá þeim sem taka þátt. Einnig verður leitað að öðrum bakteríum sem jafnan eru í nefkoki barna og ungmenna.

Hjá þeim sem samþykkja að taka þátt verður einnig óskað eftir grunnupplýsingum s.s. kennitölu, kyn, aldur og hvort viðkomandi hafi fengið bóluefni gegn meningókokkum auk símanúmers og/eða tölvupóst. Búist er við að um 0-20% þátttakenda beri meningókokka og óskum við eftir leyfi til að hafa samband við þá einstaklinga til að taka aftur sýni í 3 skipti til viðbótar þar til bakterían finnst ekki lengur (hámark 3 skipti og hámark 6 mánuðir). Allar upplýsingar eru geymdar í læstum gagnagrunni sem aðeins starfsólk rannsóknarinnar hefur aðgang að.

Ég samþykki að taka þátt í rannsókninni: Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum

Ég hef fengið og kynnt mér upplýsingar fyrir þátttakendur varðandi rannsóknina. Mér er kunnugt um að ég get dregið þátttöku mína til baka hvenær sem er.

Dagur og staður : _____

Nafn þátttakanda og kennitala: _____

☐ **Ég heimila að nefkokssýni verði tekið úr mér**

☐ **Ég heimila rannsakendum að hafa samband við mig ef meningókokkar finnast til að taka ný sýni.**

☐ **Ég heimila rannsakendum að hafa samband við mig að nýju ef niðurstöður rannsóknarinnar leiða til nýrra spurninga og leyfi vísindasiðanefndar fyrir frekari rannsókn liggur fyrir**

Nafn þess sem leggur samþykkisyfirlýsinguna fyrir:_____

Informed consent form for collection of additional swabs in the MENICE study (in Icelandic)

LANDSPÍTALI

HÁSKÓLI ÍSLANDS

Rannsóknarnúmer (t.d. M021): M_ _ _

Samþykki fyrir áframhaldandi þátttöku í rannsókninni:

Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum

Í samvinnu Háskóla Íslands og Barnaspítala Hringsins er í gangi rannsóknin: **Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum**

Í rannsókninni hafa verið tekin stroksýni úr koki hjá börnum, ungmennum og ungu fólki á Íslandi til greiningar á meningókokkum. Þetta samþykkiseyðublað er fyrir áframhaldandi þátttöku í rannsókninni. Hún felur í sér eftirfylgdarsýnatökur á þriggja til sex mánaða fresti þar til bakterían finnst ekki lengur eða til 31.12.2022, hvort heldur sem kemur á undan. Að auki óskum við eftir leyfi til þess að taka blóðprufu til rannsókna á virkni ónæmiskerfisins.

Ég samþykki að ég/barn mitt taki þátt í rannsókninni: *Algengi meningókokka í nefkoki hjá íslenskum börnum og ungmennum*. **Ég hef fengið og kynnt mér upplýsingar fyrir þátttakendur varðandi rannsóknina. Ég hef einnig fengið tækifæri til að spyrja spurninga varðandi rannsóknina. Mér er kunnugt um að ég get dregið þátttöku barns míns/mína til baka hvenær sem er**, án þess að gefa upp ástæðu fyrir því og mér er kunnugt um að það muni ekki á nokkurn hátt hafa áhrif á þá þjónustu sem mér/barni mínu verður veitt á spítalanum, hvorki núna né síðar.

Dagur og staður : _____

Nafn mitt/barns míns og kennitala:_____

Nafn foreldris/forráðamanns og kennitala (ef við á): _____

☐ **Ég heimila að sýni verði tekin úr koki hjá mér/barni mínu.**

☐ **Ég heimila að blóðsýni verði tekið til ónæmisfræðirannsókna.**

Nafn þess sem leggur samþykkisyfirlýsinguna fyrir:_____

Informed consent form for the OFICE study (in Icelandic)

UNIVERSITY OF ICELAND

Rannsóknarnúmer (t.d. O21): O_ _ _

Samþykki fyrir þátttöku barns í rannsókn:

Í samvinnu Háskóla Íslands og Barnaspítala Hringsins er í gangi rannsóknin:

Mótefnasvörun við inflúensubólusetningu hjá börnum í yfirþyngd

Í rannsókninni fá allir bólusetningu gegn inflúensu. Tekin verða blóðsýni fyrir bólusetningarnar og svo aftur 4 vikum síðar og gerðar mælingar á mótefnaframleiðslunni. Öllum verður boðið að fá deyfikrem á húðina fyrir bólusetninguna og blóðtökurnar.

Hjá þeim sem samþykkja að taka þátt verða einnig skráðar grunnupplýsingar s.s. kyn og aldur auk þyngdarmassastuðuls og fituprósentu. Allar niðurstöður verða skoðaðar undir rannsóknanúmeri og allar upplýsingar eru geymdar í læstum gagnagrunni sem aðeins starfsólk rannsóknarinnar hefur aðgang að.

Ég samþykki að barn mitt taki þátt í rannsókninni: Mótefnasvörun við inflúensubólusetningu hjá börnum í yfirþyngd

Ég hef fengið og kynnt mér upplýsingar fyrir þátttakendur varðandi rannsóknina. Mér er kunnugt um að ég get dregið þátttöku barns míns til baka hvenær sem er.

Dagur og staður : _____

Nafn þátttakanda og kennitala: _____

Nafn foreldris/forráðamanns og kennitala: _____

☐ **Ég heimila að mér/barninu mínu verði gefið inflúensubóluefni**

☐ **Ég heimila blóðsýni verði tekið úr mér/barninu mínu fyrir bólusetninguna og aftur 4 vikum síðar**

☐ **Ég heimila rannsakendum aðgang að sjúkraskrá þátttakandans (barnsins) til skoðunar á upplýsingum sem tengjast rannsókninni**

☐ **Ég heimila rannsakendum að hafa samband við mig að nýju ef niðurstöður rannsóknarinnar leiða til nýrra spurninga og leyfi vísindasiðanefndar fyrir frekari rannsókn liggur fyrir**

Nafn þess sem leggur samþykkisyfirlýsinguna fyrir:_____