Hemolytic-uremic syndrome in children in Norway – a study on epidemiology, surveillance, clinical aspects and outcome

by

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Oslo 2018

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-415-3

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

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1. Preface

Acknowledgements

I have always enjoyed science in its many forms. My parents have told me the story of the kid who entered the football pitch for the first time, only to be observed far away from play, closely studying a bug crawling around in the grass. Although those who know me would argue that I have been paying more than enough attention to the play in the years that have passed, science has followed me closely. And while bugs in the grass and books about dinosaurs have turned to medical literature, the interest in science and research has only grown stronger. I do not know what my future holds, but I am pretty certain it involves a lot of references.

This work was funded by the Norwegian Institute of Public Health and the Medical Student Research Program. I would like to express my gratitude to these institutions for making this research possible.

I would especially like to thank my fantastic supervisors who all have contributed invaluably on what is hopefully the start of a lasting journey down Research Road, in alphabetical order; Hans-Jacob Bangstad, Anna Bjerre, Knut Dahl-Jørgensen, Karin Nygård and Line Vold. To **Line** and **Karin**. Thank you for offering me the opportunity of entering into this world of science in the early years of my career. For keeping my enthusiasm high through endless rows of meetings. For invaluable advice and inspiration. To **Anna** and **Hans-Jacob (Hasse)**, who went from being the mysterious "clinicians" to becoming my supervisors. Thank you all for the patience shown, the advice given and the words of wisdom shared along the way. And a special thanks to you, **Anna**, for being my main supervisor, for words of encouragement in difficult times, for fruitful discussions, and for the patience shown. And to **Knut**, for being my supervisor throughout these final years, for the advice and for being there when I needed it.

To **Eirik Hovland**, one of my closest friends and (former) partner in crime. Thank you for the years of work we have put down in this project from the very beginning, for the endless talks we've had and for those to come. I will always have fond memories of our summer tour of Norway; collecting data, sharing laughs and enjoying the ride.

To those who have helped me in matters small and not that small, for sharing their opinions and advice; especially, but not confined to, those at the Department of Infectious Disease Epidemiology and the Women and Children's Division and the Institute of Clinical Medicine.

I would also like to express my deepest gratitude to the contributions made by the clinicians and secretaries of the pediatric departments of hospitals included in the study who assisted us greatly in the process of collecting our data.

And last, but most certainly not least – I would like to thank my family and friends for the patience, support and understanding shown during the long hours spent on this research and thesis. Special thanks to my fantastic parents who have always supported me, cheered for me, and whose contributions have made this achievement possible. I love you all and could not have done this without you!

Abbreviations

aEPEC	atypical enteropathogenic *Escherichia coli*
aHUS	atypical hemolytic-uremic syndrome
AKI	acute kidney injury
AKIN	Acute Kidney Injury Network
CA-AKI	community-acquired acute kidney injury
CDC	Center of Disease Control and Prevention
CNS	central nervous system
CRP	C-reactive protein
D^+HUS	diarrhea-associated hemolytic-uremic syndrome
D^-HUS	non-diarrhea-associated hemolytic-uremic syndrome
dL	deciliter
eae	*E. coli* attaching and effacing (gene encoding intimin protein)
EC	endothelial cell(s)
eCCl	estimated creatinine clearance
E. coli	*Escherichia coli*
ECDC	European Center for Disease Prevention and Control
eGFR	estimated glomerular filtration rate
EHEC	enterohemorrhagic *Escherichia coli*
ehxA	(gene encoding) enterohaemolysin
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EPEC	enteropathogenic *Escherichia coli*
EPO	erythropoietin
ERCP	endoscopic retrograde cholangiopancreatography
ESRD	end-stage renal disease
EU	European Union
Gb3	globotriaosylceramide 3
HA-AKI	hospital-acquired acute kidney injury
HD	hemodialysis
Hgb	hemoglobin (level in blood)
HUS	hemolytic-uremic syndrome
ICD-10	International Statistical Classification of Diseases and Healh Related Problems 10^{th} Revision
IR	incidence rate (cases per 100 000)
LD	lactate dehydrogenase
LPS	lipopolysaccharide
MDRD	Modification of Diet in Renal Disease
MLVA	multiple-locus variable-number tandem repeat analysis

MMO	Municipal Medical Officer
MSIS	Norwegian Surveillance System for Communicable Disease [Meldingssystem for smittsomme sykdommer]
MSRP	Medical Student Research Program [Forskerlinja]
NIPH	Norwegian Institute of Public Health [Folkehelseinstituttet]
NRL	National Reference Laboratory for Enteropathogenic Bacteria (at the NIPH) [Norsk referanselab for enteropatogene bakterier]
pAKI	pediatric acute kidney injury
PCR	polymerase chain reaction
PD	peritoneal dialysis
PFGE	pulsed-field gel electrophoresis
pRIFLE	pediatric RIFLE
RBC	red blood cell(s)
RIFLE	Risk, Injury, Failure, Loss, ESRD
RCT	randomized controlled trial
sCr	serum creatinine
SF O157	sorbitol-fermenting O157
SP-HUS	*Streptococcus pneumoniae*-related hemolytic-uremic syndrome
STEC	Shiga toxin-producing *Escherichia coli*
STEC-HUS	Shiga toxin-producing *Escherichia coli*-related hemolytic-uremic syndrome
STEC-LST	Shiga toxin-producing *Escherichia coli* that have lost their toxin-producing ability
Stx 1/2	Shiga toxin type 1/2
stx 1/2/AB	Shiga toxin type 1/2/common gene
TLR4	toll-like receptor 4
TMA	thrombotic microangiopathy
TTP	thrombotic thrombocytopenic purpura
US	United States (of America)
VTEC	verocytotoxic *Escherichia coli*
WBC	white blood cell (leukocytes)

List of publications

This thesis is based on the following papers:

I. **Jenssen GR**, Hovland E, Bjerre A, Bangstad HJ, Nygard K, Vold L. Incidence and etiology of hemolytic-uremic syndrome in children in Norway, 1999-2008--a retrospective study of hospital records to assess the sensitivity of surveillance. *BMC Infect Dis.* 2014 May 16;14:265.

II. **Jenssen GR,** Veneti L, Naseer U, Lange H, Vold L, Brandal LT. Implementation of multiplex PCR diagnostics for gastrointestinal pathogens linked to increase of notified Shiga toxin-producing *Escherichia coli* cases in Norway, 2007-2017. *Submitted*.

III. **Jenssen GR**, Vold L, Hovland E, Bangstad HJ, Nygard K, Bjerre A. A nationwide study of clinical, therapeutical and long-term aspects of hemolytic-uremic syndrome in children in Norway, 1999-2008. *BMC Infect Dis.* 2016 Jun 13;16:285.

IV. **Jenssen GR**, Hovland E, Bangstad HJ, Nygard K, Vold L, Bjerre A. The incidence and aetiology of acute kidney injury in children in Norway between 1999 and 2008. *Acta Paediatr.* 2014 Jul 10;103(11):1192-7.

Summary

Background: Hemolytic-uremic syndrome (HUS) is a potentially life-threatening clinica lcondition defined by impaired renal function, hemolytic anemia and thrombocytopenia. It mainly affects children of pre-school age, and is considered one of the most common causes of acute kidney injury (AKI) in children in Europe. HUS can be classified by clinical presentation as diarrhea-associated (D^+HUS) or not (D^-HUS), where the former constitute around 90 % of cases. D^+HUS is primarily caused by infection with Shiga toxin-producing *Escherichia coli* (STEC), with an estimated 5-15 % of STEC cases developing HUS (STEC-HUS) and both conditions are under epidemiological surveillance in Norway. Despite this, knowledge on HUS in children in Norway has been limited. The first national Norwegian outbreak of STEC-HUS occurred in 2006; ten children developed HUS, one with fatal outcome. This brought HUS to public attention through extensive media coverage and led to mandatory notification of all D^+HUS to the Norwegian Surveillance System for Communicable Disease (MSIS), rather than of laboratory-verified STEC-HUS only. Since the 2006 outbreak, the observed notification rate of HUS in Norway has remained relatively stable, while the number of notified STEC cases has increased markedly, especially in recent years. This has coincided with the introduction of novel diagnostic possibilities for gastrointestinal pathogens. To adjust for the increase of notified cases, the national guidelines for follow-up of STEC infections were revised in 2016, categorizing STEC as "high-virulent" or "low-virulent" based on their association with HUS.

Aims: The primary goal of this thesis was to examine and describe the central aspects of HUS in children in Norway on a national level, focusing on the areas of epidemiology, surveillance, clinical presentation and outcome. This included the surveillance of STEC, both to assess the sensitivity of HUS surveillance and the recent increase of its most common cause. Furthermore, the epidemiology and etiology of AKI in Norway was examined to assess the burden of HUS in AKI in Norway.

Materials and methods: This thesis is founded on four papers (Paper I-IV). Papers I, III and IV were based on a retrospective study of medical records of all identified HUS cases <16 years of age admitted to Norwegian pediatric departments from 1999 to 2008. Limited data was also collected AKI and nephritis cases to screen for potentially misdiagnosed HUS cases and estimate the occurrence of AKI. Paper II depicted a retrospective quality control study based on data on all STEC and HUS cases notified to MSIS from 2007 to 2017. **Paper I** described the epidemiology and etiology of HUS in children in Norway and assessed the surveillance of STEC and HUS in children through MSIS, based on extrapolation from identified HUS cases, from 1999 to 2008. **Paper II** assessed the surveillance and changes in notified STEC and HUS in all ages from 2007 to 2018 in light of implemented diagnostic measures for gastrointestinal

pathogens. **Paper III** described clinical features, therapeutic interventions and long-term outcome of the HUS cases. **Paper IV** assessed the epidemiology and etiology of AKI in Norway to examine the burden of HUS in AKI on a national scale.

Results: In **Paper I**, 47 HUS cases were identified in children in Norway in the ten year period; 38 (81%) were D^+HUS and nine (19 %) D^-HUS. The incidence rate (0.5 cases per 100,000) and proportion of cases with verified STEC infection (61 %) were low. Comparison to MSIS data showed that D^+HUS occurrence was underreported (61 %) when notification was dependent on verification of STEC. Extrapolation of numbers indicated an underreporting of STEC cases. **Paper II** depicted a significant increase in notified STEC cases and linked this to an improved capacity to detect low-virulent STEC in laboratories that implemented novel identification methods. The increase in STEC contrasted the relatively stable number of HUS cases notified yearly in and after the period assessed in Paper I. **Paper III** showed a high rate of acute renal and extra-renal complications in HUS, especially of cardiac, neurological, respiratory and gastrointestinal nature, and sepsis. The cases also had a high rate of long-term renal sequelae. **Paper IV** depicted an incidence rate of AKI (3.3 cases per 100,000 children) and found that HUS was the second most common cause of AKI in Norway (15% of all identified cases) after nephritic syndromes (44%).

Conclusions: The occurrence of HUS was higher than previously assumed, but the incidence and proportion of cases with verified STEC infection low compared to that of other countries. Meanwhile, the assessment of the sensitivity of the HUS and STEC surveillance from 1999 to 2008 showed an underreporting of D^+HUS occurrence when depending on microbiological verification of STEC, and indicated an underreporting of STEC infections. This correlated well with the increase of notified STEC in the assessment of STEC and HUS surveillance in the following years up to 2017, which was linked to gradual implementation of novel identification methods and proved to be mainly due to detection of low-virulent (non-HUS-associated, mostly non-O157) cases. These findings underline the importance of early stool sampling in suspected cases and reinforcement of mandatory notification and surveillance of both HUS and STEC. They also emphasize the importance of tailored infection control measures to hinder spread of STEC infections and decrease the burden of disease by limited follow-up of cases not associated with HUS. Furthermore, a national incidence rate of AKI (3.3 cases per 100,000 children) was estimated, although limited by the methodological approach. HUS was shown to be the second most common cause of AKI in Norwegian children. The high rate and variation of short and long-term complications in HUS cases was comparable to those of similar studies. This emphasizes the importance of close monitoring in the acute phase and thorough long-term follow-up of HUS patients. These findings may contribute to the understanding of HUS on both a national and international scale.

2. Introduction

2.1 Hemolytic-uremic syndrome – an overview

Hemolytic-uremic syndrome (HUS) was first described by Gasser *et al* in 1955 (1). It is a clinical syndrome defined by the triad of impaired renal function, non-immune microangiopathic hemolytic anemia and excessive platelet consumption leading to thrombocytopenia (2). The clinical features result from microvascular lesions termed thrombotic microangiopathy (TMA). These lesions mainly appear in arterioles and capillaries of the kidneys and the central nervous system (CNS). They include vessel wall thickening, intraluminal platelet thrombosis and partial or complete destruction and obstruction of the vessel lumina. This results in impaired vessel blood flow in the affected organs (3).

HUS is often classified by its initial clinical presentation as associated with prodromal diarrhea (D^+HUS) or not (D^-HUS). D^+HUS is most commonly caused by infection with Shiga toxin-producing *Escherichia coli* (STEC), also termed STEC-HUS. Around 90 % of HUS cases are D^+HUS (4). D^-HUS cases mainly consist of HUS caused by infection with *Streptococcus pneumoniae* (SP-HUS) and HUS associated with familiar or sporadic genetic disorders of complement regulation (5). It may also be triggered by a number of different factors, including infections, medications, defects in metabolism, pregnancy and systemic diseases (6). D^-HUS has occasionally been referred to as atypical HUS (aHUS), but the latter term is predominantly used to describe the genetic variants (7). It should be noted that aHUS episodes may present with diarrhea, for example when the HUS episode is triggered by an infection (8). Conversely, STEC-HUS presenting without prodromal diarrhea have also been reported (9). Other classifications based on both clinical associations and causalities have been suggested to accommodate for this (6;8).

HUS is associated with long-term complications such as hypertension, chronic kidney disease and end-stage renal disease (ESRD). The case fatality rate is generally considered to be 3-5 % in D^+HUS (10). Treatment has until recently been limited to supportive measures and management of complications. Eculizumab has now been proved effective in treating certain forms of atypical HUS. Eculizumab is a monoclonal C5 antibody drug which inhibits formation of the terminal complement complex (11). It has shown potential in the treatment of STEC-HUS, and trials are ongoing (12).

STEC is under epidemiologic surveillance in Norway and the EU (13;14), while HUS is under routine surveillance in some European countries (14-17). In Norway, nominative notification to the Norwegian Surveillance System for Communicable Disease (MSIS) has been mandatory for all D^+HUS and/or STEC since late 2006 and 1989, respectively. Before 2006, only notification of HUS with laboratory verified STEC infection was mandatory (13).

The estimated incidence of HUS in children has been described in several countries, although they are often based on cases notified through surveillance (Table 1). Note that some present estimates from sub-national areas, and that there are variations in inclusion age and criteria. This makes direct comparisons difficult.

Table 1: Identified estimations of annual national notification rates for HUS in children, 1993-2015.

N = cases. NR = notification rates (cases per 100 000 children). Y = years. Ref. = reference.

Country (Area)	Years included	Age	N	All HUS (NR[a])	D^+HUS (%)	<5y (%)	<5y (NR[a])	Ref.
Argentina	2004						12.2	(18)
Australia	1994-1998	<15y	98	0.64	86%	71%	1.35	(19)
Austria	1995	<15y		0.37				(20)
Austria	1997-2000	<15y		0.36			0.51	(21)
Belgium[b]	1996	<15y	38	1.8		84%	4.3	(22)
Belgium	2009-2015	<15y	110	0.8		49%	4.5	(23)
Chile	2000-2002	<15y	118	3.4		78%		(24)
England	1997-2001	<16y	287	0.71		64%	1.54	(25)
France[c]	1993-1996	<15y	286	0.70		81%	1.8	(26)
France[d]	1996-2006	<15y	961	0.71			1.87	(27)
Germany	1997-2000	<15y		0.71			1.71	(21)
Ireland	1997-2001	<16y	30	0.83		80%	2.33	(25)
Italy[e]	1988-2000	<16y		0.28	78%		0.75	(28)
Italy[f]	1997-2008	<15y	22	0.34	60%			(29)
Italy[f,g]	2003-2012	<18y	101	0.63	88%		1.57	(30)
Northern Ireland	1997-2001	<16y	16	0.97		44%	1.45	(25)
Scotland	1997-2001	<16y	63	1.56		65%	3.4	(25)
Switzerland[b]	1997-2003	≤16y	114	1.42	89%	88%		(31)
USA[f]	1994-1999	≤17y	369	0.67		71%	1.85	(32)
Wales	1997-2001	<16y	17	0.71		59%	1.49	(25)

[a] Invarialy referred to as “incidence rate” in referenced sources

[b] Including (a small number of) incomplete HUS cases

[c] Low platelet count not included in inclusion criteria

[d] Low platelet count not included in inclusion criteria. SP-HUS and Shigella-related HUS not included.

[e] Values for AKI and anemia not specified in inclusion criteria

[f] Covers a large state or region

[g] Including cases diagnosed outside of region that were referred to study center

2.2 Diarrhea-associated hemolytic-uremic syndrome

It has been argued that the classification of HUS purely based on the clinical presentation of diarrhea is insufficient. Later classifications have been more comprehensive, combining clinical features with causality (6;8). However, the D^+/D^- classification was widely acknowledged at the initiation of this study, and is still used frequently. It also reflects the previous and current notification criteria in Norway. Consequently, this classification was chosen in our study and kept for this thesis.

2.2.1 Epidemiology of D^+HUS

The most common cause of D^+HUS in children in Europe and the Americas is infection with STEC, accounting for around 90 % (2;10;18;33). However, it should be noted that STEC-associated HUS cases may more rarely present without prodromal diarrhea (9;21;28;34).

It is generally considered that 5-10 % of patients infected with STEC develop HUS (10;35). This proportion varies between studies from 1 % up to around 20 %, and more rarely 30 % (25;32;36-41). This may be explained by several factors. These studies are often based on outbreaks and/or focus on specific bacterial strains. These strains may have different virulence and potential to cause HUS. Variability in study group size may also contribute to this variation. The reported proportion of HUS is frequently higher in small STEC outbreaks than in large ones (37). This may reflect underreporting of STEC cases in small, confined outbreaks.

In Europe and the Americas, the most frequently isolated STEC serogroup in HUS patients is O157 (6;25;33;42;43). In Australia, the most isolated serogroup is O111 (19). The occurrence of isolated non-O157 serogroups has increased in later years, likely partly due to improved diagnostic tools (27;31;44). In certain regions of Africa and Asia, *Shigella dysenteriae* type 1 is considered one of the main causes of D^+HUS (45-47). This might be explained by a high incidence of *Shigella*-infections (45). Verotoxinogenic *Citrobacter freundii* has been reported in an outbreak of D^+HUS (48). A case study also identified cryptosporidium as the causative agent of D^+HUS in a 5-year-old immunocompetent child (49).

2.2.2 Risk factors for D^+HUS development

The development of D^+HUS from STEC is the net effect of several factors, including host factors and virulence profile of the bacterial strain (50). Certain properties of STEC bacteria have been associated with enhanced or lowered risk of developing D^+HUS. The presence of Shiga toxin-producing gene *stx2*, especially sutypes *stx2a* and *stx2d*, and the adherence factor intimin encoding gene (*eae*) are factors associated with an increased risk (51;52). Host factors have been described, such as low age and female gender (53). Studies have shown that human genetic factors may influence this risk. Polymorphisms known to influence the coagulation pathway have shown a strong association with development of HUS in STEC cases (54).

Studies have also associated the following factors with an increased risk of developing D^+HUS from STEC infection; bloody diarrhea, vomiting, high white blood cell (WBC) count and C-reactive protein (CRP) level in the early stages of infection, use of antimotility agents (38;53;55;56).

A debated risk factor is the use of antibiotics. Studies have concluded both for and against an increased risk of developing D^+HUS (38;56-60). It has been suggested that this depend on the type of antibiotic used. For example, β-lactams have been associated with an increased risk (53;61).

2.2.3 Clinical aspects of D^+HUS

2.2.3.1 Symptomatology – from infection to clinical syndrome

Most cases of D^+HUS are caused by STEC infection and the initial symptomatology is compatible with the typical features of infection. These are diarrhea, often bloody and/or watery, and abdominal tenderness (10;35;62;63). STEC-verified HUS cases may more rarely present without diarrhea (33).

Renal affection and the associated symptoms occur early in the development of D^+HUS. This is manifested by decreasing diuresis to oliguria or more severely anuria (64). Symptoms of extra-renal involvement may occur in the acute phase. Affection of the CNS is infrequently present, often characterized by irritability, seizures, altered consciousness and global and focal derangements (64;65). Mild gastrointestinal symptoms, such as vomiting and abdominal pain, are common. Respiratory, cardiac and symptoms related to pancreatic function are infrequently reported (66-69).

2.2.3.2 Complications – acute phase

Renal injury and failure are the most common complications in the acute phase (64). The severity of renal affection varies. It is generally considered that around 40 % of patients need dialysis (64;66). Hypertension is relatively common (70). Neurological complications are considered the main cause of mortality in the acute phase. These occur in around 25 % of cases and are often caused by multiple factors. They may be relatively mild, such as seizures and temporary neurological deficits, or more severe, such as coma, brain infarctions and edema (64;66;67;71).

Cardiac complications are less common, but an important cause of acute mortality. They include myocardial infarctions and dysfunction, pericardial effusions and cardiac tamponade, (66;72). The respiratory system may also be affected, often secondary to other factors. Complications such as pleural effusions, pulmonary hemorrhage and adult respiratory distress syndrome have been described (66;73;74).

Despite the enteropathic nature of D^+HUS, serious gastrointestinal complications are rare. These include colonic necrosis, colonic stricture formation, intussusception, rectal prolapse and oesophageal stricture (68;69;75-77). Complications involving the pancreas have been described, including pancreatitis and transient diabetes. Involvement of the biliary system, with hepatic cytolysis and

cholelithiasis, are also known to occur (68;78). Ocular involvement is rare and vary in severity, but may present as pronounced retinopathy with retinal ischemia (79).

2.2.3.3 Complications – long term

Long-term complications of D^+HUS mainly consist of renal sequelae. This manifests as reduced glomerular filtration rate, hypertension and/or proteinuria. The occurrence varies between studies (64). In a large meta-analysis by *Garg et al.*, it was estimated that 25 % of patients had renal sequelae and an additional 3 % had progressed to ESRD at a minimum of 1 year of follow-up (80). Another study showed prolonged hypertension in 15 % of cases, chronic renal failure in 14 % of cases and a cumulative incidence of ESRD of 3,6 % (81). Renal sequelae may also develop years after clinical recovery (82). One study has shown that screening for microalbuminuria in the long-term follow-up of HUS patients increases the sensitivity for predicting the occurrence of such cases (83).

Extra-renal long-term complications are rare. Prolonged, recurring and post-recovery developed diabetes has been described (84;85). Persistent neurological damage has been reported, such as cortical blindness, choreatic syndrome and late secondary sensorineural hearing loss (67;86). Long-term ocular complications are very rare, but serious affection such as neovascularization and subsequent optic nerve atrophy has been described (79).

A study on STEC and STEC-HUS patients from the 2011 outbreak in Germany investigated psychological outcome, fatigue and quality of life compared to the general population, six months after initial infection (87). Thirty-one percent of the study patients had developed HUS. They reported that the STEC/STEC-HUS patients suffered from substantially elevated self-reported levels of depression, post-traumatic symptoms, fatigue, anxiety and impaired quality of life. Numbers were not markedly higher when compared to patients who have survived other major illnesses. Nevertheless, 3 % of patients met the criteria for posttraumatic stress disorder and 15 % for major depressive disorders. This implies that potential long-term effects on mental health warrant attention in the follow-up of D^+HUS patients.

2.2.4 Prevention of D^+HUS development

No proven treatment options exist to prevent development of D^+HUS from STEC (88). Volume expansion therapy with isotonic solutions in the early phase of STEC diarrhea have shown some effect in reducing oligoanuria in the acute phase of D^+HUS (89;90). The only effective measure available is hence to prevent the causal infection (91;92). This includes a variety of measures aimed at proper handling of food products from industrial production to household preparation, basic hygiene related to contact with food and animals and isolation of affected patients.

The use of antimicrobial agents in manifest STEC infection has been controversial. Studies suggest that certain antibiotics increase the chance of developing

D^+HUS by enhanced toxin gene expression and release or production of toxins (93). Other studies indicate that this depend on the bacterial strain and type of antibiotic used (61;94). Current reviews advise against the use of antibiotics in STEC infections (60;88;95), although recent publications have called for a more nuanced approach. They propose the use of specific antimicrobial agents within certain limitations (61;96;97). Conversely, antibiotics are considered necessary in infection by *Shigella* species (98).

2.2.5 Treatment of the clinical syndrome

No curative treatments have proven safe and beneficial for D^+HUS (99). The management of D^+HUS is primarily focused on supportive therapy and treatment of complications (100).

Hemolytic anemia is one of the defining features of HUS. Around 80 % of patients receive red blood cell (RBC) transfusion during admittance (66;101). It has been suggested that insufficient erythropoietin (EPO) synthesis may aggravate the hemolytic anemia in D^+HUS (102). A randomized pilot trial showed potential in early administration of EPO to reduce the need for RBC transfusions (103). However, a recent case control study found no effect (104). This has yet to be assessed in larger trials.

Consumption of thrombocytes leading to thrombocytopenia is an important feature of HUS. Platelet transfusions are usually avoided as they might increase microthrombi formation and promote tissue ischemia (105). However, a recent case-control study showed no difference in patient outcomes for those receiving and not receiving platelets. This suggests that individual assessment is necessary for patients with severe thrombocytopenia (101;106).

The third defining feature is acute kidney injury with varying degrees of renal insufficiency. Control of fluid status and electrolyte balance, monitoring for and treatment of hypertension and dialysis treatment when required, remain the cornerstones of available interventions (100). It is debated whether peritoneal dialysis (PD) or hemodialysis (HD) is the most beneficial modality in D^+HUS. PD tends to be the preferred option in literature, although the clinical conditions define the modality; PD can be difficult to perform when a patients presents severe gastrointestinal symptoms (21;101;107).

The use of plasma exchange therapy in D^+HUS is also debated. Plasma exchange therapy was recommended in the 2013 Guidelines of the American Society for Apheresis, despite acknowledging the lack of evidence of therapeutic effect (108). Some studies show no effect of plasma exchange (100). Plasma infusion present an option, though with a risk of fluid overload, and is not recommended as first line treatment in D^+HUS (108).

Steroids, anticoagulants (heparin) and fibrinolytics have no benefit in D^+HUS treatment (100). Antimotility agents are contraindicated (33). The use of diuretics is

generally avoided to treat hypertension in the acute phase due to the risk of further aggravating dehydration (109).

In severe cases where the kidney injury progresses to ESRD, renal transplantation is the last option. This is considered safe in D^+HUS patients, while it is conversely associated with a high relapse rate in atypical HUS (110).

2.2.6 Potential future treatments

Although no curative treatment options have proven effective in D^+HUS, certain advances have been made.

The most promising drug to date is eculizumab. Eculizumab is a terminal complement inhibitor that has proven effective in some forms of atypical HUS (11). Research has in recent years indicated that complement activation plays an important role in STEC-HUS pathogenesis (111;112). Eculizumab has shown variable results in D^+HUS, but trials have mainly been performed in small patient groups. Promising results have been shown in severe cases and in improving outcome in cases with CNS affection. Trials are ongoing to evaluate the effectiveness of this treatment (12;67;113;114). The safety and duration of extensive treatment with a complement inhibitor is also being addressed. . There may still be some time before eculizumab is a recognized intervention in D^+HUS patients (88;110).

Therapies targeting Shiga toxins (Stx) and prevention of their activity are also being developed. Treatment with monoclonal antibodies against Stx2 (115) has been approved as an orphan drug in both Europe and USA (110). Sorbents designed to bind and neutralize Stx have been tested through an RCT, albeit with disappointing results (116). Removing Stx and anti-Stx antibody-formed immune complexes by IgG depletion through immunoadsorptoin has shown promising results in patients with severe neurological complications (117). Intramuscular injection of adenovirus vector expressing Stx1/2-neutralizing agents has shown similar effects in animal models (118). Mice studies have shown that specific tetravalent peptides inhibit Stx cytotoxicity by high affinity binding to and neutralization of the toxins (119). Research to further evaluate these therapies is ongoing (88;110).

The use of recombinant, human, soluble thrombomodulin α, exerting anticoagulatory and anti-inflammatory effects on endothelial cells, has been reported successful in the treatment of three patients (120). Another mice study has indicated that the antimicrobial peptide cathelicidin plays an important role in lowering the susceptibility to STEC O157:H7 infection. This suggests that administration or stimulation of production of the peptide may be useful in future treatment of STEC-HUS (121).

2.2.7 Prognostic factors in D^+HUS

HUS associated with diarrhea and/or STEC infection has a favorable renal prognosis compared to aHUS (81). The most reliable predictor of recovery of renal function is a

short duration of oliguria/anuria in the acute phase. Factors associated with a worse long-term prognosis are the severity of acute illness, neurological involvement in the acute phase, a high WBC count with neutrophilia, high serum creatinine (sCr) or urea concentration, hypertension, ischemic colitis, increased CRP and increase of certain interleukins (80;122). Despite the strong association between the severity of illness and a worse prognosis, long-term complications are also seen in cases with a mild acute phase (80).

A high WBC count is considered indicative as a predictor of severe disease. A positive correlation has been shown to mortality, anuria, need for and duration of renal replacement therapy (RRT) and neurological involvement (122;123).

Studies have shown that certain genetic factors are associated with a prolonged need for RRT. This applies especially to specific genetic factors influencing the coagulation pathway (54).

2.3 Shiga toxin-producing Escherichia coli

STEC can be defined as a group of pathogenic strains of *Escherichia coli* (*E. coli)* bacteria harboring genes coding the production of Shiga toxins (Stx that may potentially cause illness in humans. Enterohemorrhagic *E. coli* (EHEC) can be defined as STEC that causes hemorrhagic colitis in humans. All STEC are not necessarily pathogenic to humans. The term EHEC is therefore often used for the subgroup of STEC that is highly associated with disease in humans, and may be referred to as humanopathogenic. These terms are often used interchangeably. Notification criteria in Norway use the term EHEC (124). STEC is now widely considered the preferred term in infectious disease epidemiology and surveillance (10;35). STEC is used throughout this thesis to avoid confusion and adhere to current epidemiological terminology trends, regardless of terminology used in the cited literature.

STEC was first described in association with an outbreak of O157:H7-related hemorrhagic colitis in the United States in 1982 (125). Isolation of the same serotype in a sporadic case from 1975 was mentioned in this paper. Later in 1982, the production of Stx was shown in strains of *E. coli* known to cause diarrhea. These toxins were found to be similar to those of *Shigella dysenteriae type 1* (126). The following year, the same toxins were detected in stool isolates of *E. coli* from children with sporadic HUS. The Stx were shown to be toxically active on Vero cells (green monkey kidney cells), suggesting an association between STEC and HUS (127). This cytotoxic effect differed from the established properties of *E. coli* enterotoxins and had already been described in studies published in 1977 (128). The toxic effect on Vero cells gave rise to the term verocytotoxic *E. coli* (VTEC). VTEC is used interchangeably with STEC (35).

2.3.1 Epidemiology of STEC

In 2014, Majowicz *et al* published a paper estimating a global incidence of STEC-related illness (129). They estimated that STEC causes 2 801 000 cases of acute illness annually. This estimation was subject to certain limitations, but indicated STEC as a global health issue. Since it was first described in 1982, STEC has emerged as a health threat in both developed and developing countries and regions worldwide. In many, the true extent of the disease burden remains unknown (19;130-135).

The epidemiology of STEC in the European Union (EU) is well described. National surveillance results are reported to ECDC from the memberstates, Norway, Switzerland and Iceland and summary reports are published yearly by the European Centre for Disease Prevention and Control (ECDC) in collaboration with the European Food Safety Authority (EFSA). In 2009, 3573 cases of STEC infection were reported, half of which were of STEC O157 origin (44). This increased to 9485 in 2011, strongly augmented by the outbreak originated in Germany (136). In 2013, 6043 confirmed STEC cases were notified. The notification rate was 1.59 cases per 100 000 population, 5,9 % higher than in 2012 (132). The highest notification rates were seen in Ireland, Netherland and Sweden, with 12.3, 7.1 and 5.8 cases per 100 000 population, respectively. The lowest notification rates (<0.1 cases per 100 000 population) were seen in Bulgaria, Cyprus, Greece, Latvia, Poland, Romania and Spain. It should be noted that these numbers dependen on several factors, from doctorseeking behavior and available laboratory technices in the memberstates to the true incidence of the disease. Notification rates from the different memeberstates should therefore be interpreted with caution.

The Centers for Disease Control and Prevention (CDC) estimate that around 265 000 human STEC infections occur each year in the United States (US) (35). In 2015, the population-based Foodborne Disease Active Surveillance Network report covering 15 % of the US population addressed the incidence trend from 2006-2014 (131). The incidence had decreased for STEC O157 and increased for non-O157 STEC infection in 2014 compared to 2006-2008. The 2014 incidence was estimated to 0.92 and 1.43 cases per 100,000 in the overall population for O157 and non-O157, respectively. In the report, the increased incidence of non-O157 infection was attributed in part to improved laboratory recognition and identification. This was further reinforced in the 2018 report, which showed that the incidence of non-O157 had further increased by 25 % while the O157 incidence remained stable compared to 2014-2016 (137).

Nominative notification of all STEC cases to MSIS has been mandatory since 1989 (124). STEC epidemiology in Norway will be discussed later (see 2.7).

The various STEC serotypes have different epidemiology. STEC O157:H7 is the most commonly isolated serotype in human STEC infections in Europe and the Americas. It is an emerging pathogen in Africa since it was first isolated in a large

outbreak in 1992. The frequency of isolated non-O157 pathogens has increased in recent years, partly due to improvements in diagnostic procedures and detection methods (21;44;135;138;139). In Australia, non-O157 serotypes are most commonly identified, particularly serogroup O111 (19).

There is a clear seasonal variation in human STEC infections. Studies have shown a peak in summer months and early fall (July-September), although occurrence in Europe remain above average in winter months (140).

2.3.1.1Animal reservoirs for STEC

Cattle and other domestic ruminants are natural reservoirs of STEC (141). This applies to both O157 and non-O157 strains (142). Cattle are normally asymptomatic carriers and shed the bacteria via feces. However, STEC has also been shown to cause severe diarrhea and induce intestinal damage in cattle, especially in calves (143).

There are coinciding factors between bovine STEC prevalence and occurrence of human STEC infections. Both show the same seasonal variation, peaking in summer months and early fall (140;144). Studies have found an association between geographical cattle density and incidence of human STEC infection (145-147). This association has also been shown in relation to incidence of pediatric HUS (148).

Cattle density is thus identified as a risk factor for contracting STEC. Other related risk factors are farm visits, contact with animals or animal manure, eating undercooked meat and contact with recreational water (149;150).

Most studies on occurrence in animals have been conducted in cattle. In a number of countries, including Australia and Norway, sheep are considered important reservoirs. Studies in Norwegian farms have identified the bacteria in both sheep and cattle (151;152).In addition to cattle and sheep, STEC O157:H7 has been isolated from a wide range of animals, including pigs, pigeons, bison, deer, sea gulls and fish (139;152-156).

2.3.1.2 Transmission of STEC to humans: from sporadic cases to outbreaks

The estimated infectious dose required to develop clinical STEC infection is low (157). The incubation period is around 3-4 (1-10) days (10;62). STEC can be transmitted to humans in several ways. The most common is foodborne transmission through contaminated food products. Meat products were the first products associated with STEC outbreaks following two outbreaks in 1982 (125). The colloquial term “the hamburger bacteria” was later introduced in the press after an outbreak of O157:H7 in the US in 1992-1993 (158). Consumption of meat products is an identified risk factor for STEC-associated disease. The potential for contamination exists throughout the production process from farm to fork. This has been shown in the production chain of beef, where a large review study found a prevalence of *E. coli* O157 of 1.2 % in sampled raw beef products (144). Several meat products have been associated with STEC infections. These include ground beef patties (hamburgers), pork, crab meat,

deer meat (jerky), dry fermented salami, semidry fermented sausage (mettwürst), and cured mutton sausage (157;159-164).

Other food products associated with transmission to humans include vegetables and fruits. This is often due to contamination by manure during irrigation or harvesting, or poor storage and insufficient preparation of products. Certain pesticide solutions have even been indicated as having a stimulatory effect on *E. coli* O157:H7 (165). STEC contamination has been identified in a wide range of vegetables and fruit. These include strawberries, watercress, spinach, apple cider, romaine and leaf lettuce, white radish, alfalfa and bean sprouts. (40;41;166-172). Intake of unpasteurized milk and milk products are also common sources of human infections (173-175).

New and previously unseen food products have been implicated as vehicles of transmission in recent years. Hazelnuts were identified as the source in a multistate outbreak of O157:H7 in USA in 2010-2011 (176). Prepackaged cookie dough was associated with another US outbreak of STEC O157:H7 in 2009 (177). An outbreak in Japan in 2011 was strongly associated with consumption of rice cakes (178). Contamination had most likely occurred during the manufacturing process. These findings imply that STEC infection can stem from any food product in the absence of proper precautions.

Drinking or swimming in contaminated water has been associated with human infections (135;179;180). STEC can also be transmitted to humans through direct contact with animals. Outbreaks originating from establishments where visitors have direct contact with animals have been reported (181).

Interpersonal transmission has been thoroughly described. It usually occurs in facilities and situations where close contact between subjects is common. This includes infection transmitted by family members. Outbreaks infrequently occur in day-care centers, nursing homes and similar institutions. There these subgroups are gathered and exposed to both interpersonal transmission and the same potential sources of infection (meals, water, etc.) (182-184).

According to the CDC, around 80 % of STEC cases are sporadic and 20 % part of recognized outbreaks in the United States (35). This correlates with studies and surveillance data from Europe showing that most HUS-related STEC cases occur sporadically (27). Large outbreaks of STEC do occur, and can have a serious impact on public health. Outbreaks occur worldwide, their geographical distribution and severity varies and they often attract massive media attention. Examples of important national and international outbreaks are listed in Tables 2 and 3. The table depicts year(s) of occurrence, country of origin, bacteria serotype or -group and number of cases that developed HUS.

Table 2: Verified local and national outbreaks of STEC infection in Norway, 1999-2017

Year	Type	Serotype	STEC / HUS cases	Ref.
1999	Local	O157:H7	4/0	(13)
2003	Local	O157	5/0	(185)
2006	National	O103:H25	17/10[a]	(163)
2009	National	SF O157	13/9	(13)
2009	National	O103	7/0	(185)
2009	Local	O145:H?	3/3	(185)
2009	Local	O121:H19	3/1	(185)
2009	Local	O145:H28	16/0	(185)
2009	Local	O?	4/0	(185)
2010	Local	SF O157	3/3	(13)
2013	National	O157	11/4	(185)
2013	Local	O145	6/3	(185)
2015	Local	O157:H7	11/0	NP[b]
2017	Local(?)	O157:H7	4/1	NP[b]
2017	Local	O157:H7	3/2	NP[b]
2017	Local	O157:H7	4/0	NP[b]

[a]One included case was later identified as O157:H7, but was kept as an outbreak case according to case criteria.

[b]NP = not pulished.

Table 3: Examples of verified large national and international outbreaks of STEC infection

	Large national and international outbreaks			
1982	USA	O157:H7	47/0	(125)
1989-90	USA	O157:H7	243/2	(179)
1990	Japan	O157:H7	174/14	(39)
1992	Swaziland	O157:NM	Thousands/unknown	(135)
1992	Italy	O111:NM	Unknown/9	(186)
1992-3	USA	O157:H7	501/45	(158)
1995	Australia	O111:NM	Unknown/21	(164)
1995-6	Sweden	O157 (4 subtypes)	110/29	(187)
1996	Japan	O157:H7	8576/106	(168)
1996	Central African Republic	Non-O157:H7 (Stx2 verified)	(Confirmed in) 86/several	(188)
1996	Scotland	O157:H7	345/34	(189)
2002	Germany	O157:NM	Unknown/38	(190)
2005	France	O157:H7	69/17	(191)
2006	USA	O157:H7	205/60	(41)
2006	USA	O157:H7	77/7	(192)
2008	USA	O157:H7	99/3	(193)
2008-9	The Netherlands	O157:NM	20/0	(194)
2010-11	United Kingdom[a]	O157:H7	252/2	(195)
2011	USA	O157:H7	58/3	(172)
2011	Germany (origin)	O104:H4	3816/845 (Germany)	(40)
2011	Japan	O157:H7	167/5	(178)
2012	Denmark	O157:H7	13/8	(52)
2013	Italy	O26:H11	?/20	(196)
2016	United Kingdom[a]	O157:H7	165/9	(197)

[a)] 2010-11: England and Wales. 2016: England, Wales and Scotland.

2.3.2 Clinical manifestation and complications

Clinical symptoms of STEC infections vary. Severity ranges from mild to life-threatening. The clinical presentation usually includes one or more of the following; watery or bloody diarrhea, abdominal pain/cramps vomiting and more rarely low-grade fever (10;35;62). STEC may also be identified in asymptomatic cases (63). Patients suffering from self-limiting STEC gastroenteritis may show temporary impairment of renal function due to dehydration. This is not to be confused with long-term renal sequelae associated with development of HUS (198).

An estimated 5-10 % of STEC cases develop HUS. This depends on both host and bacterial factors and is described later. STEC infection may in rare cases lead to thrombotic thrombocytopenic purpura (TTP). In both HUS and TTP, thrombotic microangiopathy (TMA) is the central pathophysiology feature. It is still debated whether HUS and TTP are different syndromes or represent a spectrum of the same disease. There are certain acknowledged differences. TTP is mainly caused by plasma deficiency of the von Willebrand factor cleaving protease called ADAMTS13. This is due to genetic mutations or autoantibodies and is a distinct disease entity. The deficiency may lead to platelet aggregation if triggered by for example an infection (8;199;200). TTP occurs less frequently than HUS in children. Both may present with varying degrees of CNS and renal involvement, but the former is predominantly seen in TTP (98). The conditions are often initially difficult to distinguish in a clinical setting. However, early differentiation is important. One reason is that early plasma exchange have proven effective in the former, but not the latter (100). Diagnostic tests to measure ADAMTS13 activity may in such cases contribute to a rapid diagnosis (201).

2.3.3 Prevention of STEC infection

Increased knowledge on contamination of food products has led to extensive research targeting preventive measures. Control measures have targeted different stages in the food-production chain. Implementation of proper hygienic measures throughout this production chain has been essential.

Outbreaks related to drinking water invariably occur in developed countries despite continuous improvement of water decontamination (132). Safe supply of treated and clean water is absent in large parts of the developing world. Low-cost public health intervention strategies include providing suitable water containers, water disinfectants and public education. These are among the solutions implemented in an effort to reduce human exposure to waterborne pathogens in developing countries (202).

Measures to reduce occurrence of STEC in animal populations have been investigated. Methods are being developed to reduce the risk of transmission from contaminated sources (soil, plants, etc.), either by reducing the presence of bacteria or the susceptibility to infection. One recommended method is addition of hydrated lime

to soil contaminated with STEC (here O157:H7). This has been shown to reduce the bacterial count to below the detection limit (203). Another method under development is passive immunization against STEC O157:H7 by vaccination of cattle (204).

Measures exist that are aimed at reducing established STEC colonization and thus faecal shedding of bacteria. The use of hybridized antimicrobial drugs based on organic salts (GUMBOS) has shown some effect in reducing STEC transmission and cytotoxic activity (205).

The use of bacteriophage treatment is showing promising results in already established contamination of food products. This remains an experimental treatment (206). High pressure treatment of heat sensitive food products inoculated with different STEC strains has shown significant effect in a controlled setting (207). Chemically based treatments are also being explored. These are challenging due to the development of resistance towards the chemicals used. An alternative is the use of bacterial nutrients that manipulate bacteria, rendering them less harmful to humans. Two such nutrients, acetoacetic acid and beta-phenylethylamine, have been shown to drastically reduce bacterial cell numbers in beef meat (208). Certain plant extracts have shown effect in inhibiting biofilm formation and decreasing STEC cell adhesion to human epithelial cells (209).

Measures are being developed to directly prevent infection in humans. Mice trials have been performed using vaccines providing immunity against certain surface polysaccharides that are produced by many bacteria, including several common STEC serotypes. Results have shown that these may contribute to protection against STEC infection (210).

An important prevention strategy is to implement measures to prevent interpersonal transmission. These include restrictions to isolate or exclude the infected person from situations with increased risk of transmission (e.g. work, nursery homes, etc.) and postdiarrheal fecal sample controls (211).

2.4 Detection of STEC infections

When an STEC infection is suspected, a quick and targeted diagnostic process is necessary. Confirmation affects immediate medical decisions and is crucial for a proper investigation to identify the source in the event of an outbreak and early implementation of appropriate control measures. STEC detection has historically been focused on O157:H7. The shortcomings of this approach have become increasingly apparent throughout the last two decades. Research now show that non-O157 STEC likely causes more than half of human infections (212). Continuous improvements in diagnostic procedures have contributed to a notable increase in identified non-O157:H7 STEC (21;27;31;44;51;138).

The ECDC updated the European case definitions for STEC in 2012; a verified STEC requires confirmation of Stx or Stx genes (*stx*), except when STEC O157 is

directly isolated (42). Various methods are available to identify the presence of STEC; presented below are some of those most commonly applied.

2.4.1 Fecal culturing

Fecal culturing remains one of the hallmark methods for STEC detection as it also provides bacterial isolates for further characterization (212). Fecal samples for culturing should be secured from the patient as early as possible after the onset of diarrhea.

One of the most commonly applied fecal culturing techniques consists of culturing bacteria on sorbitol MacConkey agar inoculated with a fecal sample (213). This is a simple and low-cost method used to detect the most commonly identified STEC, O157:H7. STEC O157:H7 is unable to ferment sorbitol, unlike most other *E. coli* strains. It is recognizable as a gray colony, in contrast to sorbitol-fermenting pink colony strains. Enrichment on broth and immunomagnetic separation using anti-O157 coated magnetic beads before plating further enhances the sensitivity of the method (213;214). Chromogenic medium, such as CHROMagar O157, is an example of other medium used to culture O157 (215). Both CHROMagar and MacConkey agar have the disadvantage of not detecting most non-O157 (212).

A range of alternative agar media have been developed following the emerging importance of non-O157 STEC (212). These may be selective for non-O157 serogroups, such as chromogenic media (CHROMagar STEC and CHROMagar STEC O104) (216), others are capable of identifying both O157:H7 and non-O157 STEC (212;216).

Culture-based methods remain important in STEC detection. However, there are drawbacks such as their limits in sensitivity for certain STEC and the time consuming process involved (212). Therefore, supplementary assay types are recommended for a more effective confirmation of STEC (217).

2.4.2 Enzyme immunoassays

Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) tests are rapid and sensitive method for direct detection in stool samples or enriched cultures (212). They may be used on samples to detect antibodies for Stx or various STEC components, such as serotype specific lipopolysaccharides (LPS) (218-220). They may also be applied to detect the STEC O-groups of certain serogroups in food samples (221).

2.4.3 Polymerase chain reaction

Molecular methods, such as DNA hybridization and polymerase chain reaction (PCR) techniques, commonly use nucleic acid-based techniques to detect STEC by targeting Stx and STEC specific genes (212). Specific PCR assay panels may be used to detect and differentiate between coding sequences of *stx1*, *stx2* and other common virulence genes such as *eae* in fecal samples. Detecting both *stx* and other virulence factors is

important, as the ability to produce Stx may be lost either *in vivo* or *in vitro*, rendering the bacteria undetectable to *stx* PCR only (124;222). The high sensitivity and specificity and rapid detection of real-time PCR makes it an ideal screening tool in suspected STEC cases compared to other methods (223). Recent years have also seen the development of various multiplex PCR assays (in this paper referred to as "broad screening PCR") that may simultaneously screen for multiple enteropathogens including STEC (212).

2.4.4 Pulsed-field gel electrophoresis and multiple-locus variable-number tandem repeat analysis

The increasing multitude of STEC has highlighted the need for efficient tools to link isolates on an epidemiological level for further improvement of related public health measures. This has contributed to the development of methods to further characterize isolates through genotyping. Pulsed-field gel electrophoresis (PFGE) has historically been considered the gold standard because of its high discriminatory power and value in epidemiological work (212). Multiple-locus variable-number tandem repeat analysis (MLVA) has since emerged with increasing use. MLVA has capabilities comparable to PFGE, but is simpler and less expensive. They may also be combined for enhanced results. PFGE has the advantage of using a reliable electronic database for exchange and comparison of STEC strain profiles (PulseNet International protocol) (212;224).

2.4.5 Whole genome sequencing

Whole genome sequencing (WGS) has in recent years emerged as the superior alternative in terms of genotyping methods (212). Since its introduction, the timeliness and associated costs have been reduced enough for it to provide faster results at a lower cost than earlier and more complex diagnostic routines (225). WGS allows for analysis of the entire genome rather than limited elements and show better discriminatory power versus previous options (212). Consequently, one of the main challenges with WGS is standardization, management and storage of the vast data generated for bioinformatic analysis. This is important to enable comparison between isolates in a surveillance setting. Various computing methods and online tools exist to facilitate this process (212). There are also numerous intiatives and projects currently ongoing to enhance collaboration on this across sectors and borders.

WGS generate raw genomic data that may be analyzed directly or assembled as draft genomes using reference genomes. This can be applied for comparison to other genomes to identify similar pathogens using a range of computing approaches targeting different genetic aspects of the isolates. Selected methods are assembled in "pipelines", where genomic data may be analyzed using preset software algorithms (226). This also renders WGS capable of replacing common STEC serotyping and *stx* subtyping (212).

2.5 Pathophysiology of STEC-mediated HUS

It is generally considered that around 90 % of HUS cases are diarrhea-associated. The majority are caused by STEC infection. STEC infection has also been shown to cause HUS without prodromal diarrhea. The pathophysiology of STEC-mediated HUS thus accounts for the pathological process seen in most HUS cases. In this part of the thesis, the path from infection to developed HUS will be assessed. This is made possible by the potent weapons of STEC bacteria – its virulence factors.

2.5.1 STEC virulence factors

Some *E. coli* species are naturally harbored in human intestines. Others have animal reservoirs and enter the human body through the fecal-oral route. Most are harmless to humans, but some carry with them the potential to cause disease. And while these often result in mild gastrointestinal symptoms, some possess virulence factors associated with severe disease. STEC are among the pathogenic harboring virulence factors that enable them to cause serious illness in humans. STEC are generally not considered invasive pathogens, but some of their virulence factors provide access to the circulatory system where they may inflict severe invasive damage (35;124).

2.5.1.1 Shiga toxins

Shiga toxins (Stx), formerly known as Shiga-like toxins are considered the most important virulence factor in STEC strains. Stx are divided into toxin families. Two major families have been identified; Stx type 1 (Stx1) and Stx type 2 (Stx2). These are further divided into different subtypes based on their protein structure (227).

The two corresponding major toxin gene types (*stx1* and *stx2*) and their subgroups differ in their association to the development of HUS (51;55;228). *Stx1* alone is rarely identified in STEC-HUS and has even been associated with a reduced risk of HUS development (55). The presence of *stx2* has been associated with an increased risk of HUS and a more severe disease progression (228;229). This association was stronger in subtypes *stx2a*, *stx2c* and *stx2d* (51;55).

The toxin genes (*stxAB*) are located in the genome of bacteriophages, making them mobile elements. There is one phage for each toxin variant. Bacteria can have more than one phage, thus producing different toxins (230;231).

Stx are part of a larger toxin family called AB_5 toxins. These are characterized by their structure, consisting of an A subunit with enzymatic activity and a B subunit pentamer. The B subunit interacts with glycolipid receptors (globotriaosylceramide 3; Gb3) on certain eukaryotic cells, especially in the colon, kidney, brain and pancreas. This mediates receptor-dependent internalization and transport to the endoplasmatic reticulum. There the A subunit of Stx (as opposed to other AB_5 toxins) cleave ribosomal RNA, which in turn inhibits protein synthesis and causes cell death (232). They may also induce cell death by apoptosis (233).

The toxigenic effect of Stx has been shown to induce expression and release of proinflammatory cytokines in some cells. This may lead to upregulation and expression of glycolipid receptors in other cells, promoting further toxin interaction (233).

An in vivo study by Bentancor *et al* using mice has demonstrated that eukaryotic cells cloned with the *stx2* gene could express Stx2 (234). This showed cytotoxic activity in plasma and reproduced the pathogenic damage seen in Stx verified STEC infection. The results suggest that local eukaryotic cells might be an alternative source of toxin production in STEC-mediated disease.

2.5.1.2 Other virulence factors

Lipopolysaccharide (LPS) is the major outer membrane component in Gram-negative bacteria, including STEC. LPS consists of three components; a core oligosaccharide, the biologically active lipid A and a polysaccharide side chain. The latter is referred to as the O-antigen. It projects from the membrane surface and varies in structure between bacterial species and strains (235;236). Lipid A interacts with toll-like receptor 4 (TLR4), which is expressed on leukocytes, platelets and potentially other cells through stimuli response. This may initiate pro-coagulant activity leading to activation of leukocytes and platelets and subsequent proinflammatory cytokine expression and platelet consumption (236-238).

An important factor contributing to STEC virulence is the ability to adhere to intestinal epithelium, called attaching and effacing activity, an ability related to outer membrane proteins. One of the most prominent proteins is intimin (EaeA), which is encoded by the *eae* gene. Other important adhesion enhancing proteins are the *E. coli* secreted proteins (Esps) A, B and D. Some are also found in enteropathogenic *E. coli* (EPEC). The genes coding for these attaching and effacing proteins are located on pathogenicity islands called locuses of enterocyte effacement (239).

STEC also possess virulence factors in the shape of bacterial plasmids. They are self-replicating, extrachromosomal elements that promote dissemination of certain bacterial traits, including virulence, metabolism and resistance to antimicrobial agents (240).

Other molecules have been identified. The contribution to STEC pathogenicity is not fully known in most of them. Two toxicogenic virulence factors of increasing interest are hemolysin and a new AB_5 cytotoxin called subtilase. Both have shown an ability to trigger epithelial and/or endothelial cell apoptosis and microvascular thrombosis in laboratory conditions (241;242).

2.5.2 Intestinal involvement

STEC bacteria are ingested and pass through the acidic barrier, reaching the ileum. There they bind to villi and the follicle-associated epithelium of Peyer's patches. STEC then colonize the mucosal epithelium of the colon. Some strains have adapted

by previously passing through the gastrointestinal tract of a host of the same species, so-called horizontal transfer. These may generate a more persistent and generalized colon colonization (231;243).

STEC adhere to the epithelium through intimin and secreted bacterial proteins (239). Precisely how the bacteria contribute to intestinal pathology is uncertain. Gb3 receptors are present in intestinal epithelium, and both receptor-dependent and independent uptake in colonic epithelial cells has been shown as potential pathways through the intestinal barrier. Studies indicate that Stx2 mediates functional cell alterations and apoptosis independent of uptake mechanism. This may interrupt the balance of intestinal absorption and secretion, causing hemorrhagic fluid accumulation in the colon (231;233).

2.5.3 Systemic involvement

Certain blood components have been implicated in the process where virulence factors gain access to and are transported in the circulation. Stx has been shown to move proportional to transmigration of neutrophils across the colonic epithelium. Both Stx and LPS are present in the circulation in STEC-HUS, bound to monocytes, neutrophils and platelets, either alone or as complexes. Unbound Stx has not been verified in serum. How Stx is further transferred to human endothelial cells is not fully known. Suggested mechanisms include differences in receptor binding affinities of target tissues (231;237;238;243).

2.5.4 Microvascular injury

STEC virulence factors may affect most vasculature. Damage is predominantly seen in small vessels in the kidneys, digestive tract, and the CNS, all expressing the Gb3 receptor. These vessels are considered more sensitive to the given concentration of Stx (8;232).

Interacting through the Gb3 receptor, Stx activates and exerts direct damage on the endothelial cells (EC) and induces a proinflammatory response. The result is enhanced local leukocyte adhesion and a local prothrombotic state. Other virulence factors contribute to the inflammatory response and increase the EC susceptibility to Stx damage. Host responses also contribute to this development. This is likely mediated through the release of proinflammatory mediators and recruitment of tissue-specific Stx-bound leukocytes. This includes activated neutrophils that degranulate and release reactive oxygen species and proteases that may induce local cell injury (231;243).

The local prothrombotic state results from secretion of thrombotic factors that activate the coagulation cascade and inhibits fibrinolysis (231). Combined with direct EC damage, the result is abnormally increased shear stress within the small vessels. This leads to platelet aggregation on the activated EC and subsequent mass consumption, manifesting as thrombocytopenia and formation of fibrin-rich

microthrombi. The increased shear stress and inhibited fibrinolysis likely result in continuous destruction of RBCs. This presents as the distinctive feature hemolytic anemia, although the exact mechanism of hemolysis is unclear (8;243).

The vessel wall thickening, intraluminal platelet thrombosis and partial or complete destruction of the small vessel lumina lead to impaired blood flow in the affected organs. Diminished blood flow may in turn create ischemic changes and mild to severe organ tissue damage (3).

2.5.5 Renal involvement

The primary organ affected in HUS is the kidneys, more specifically the renal endothelium. These cells are especially sensitive to Stx interaction due to their high expression of Gb3 receptors. A high sensitivity to Stx has also been shown in podocytes and mesangial cells in renal glomeruli. Gb3 receptor expression has also been shown in extraglomerular tubular cells (243).

Stx action on renal EC usually manifests by endothelial swelling and detachment of cells from the basement membrane due to subendothelial swelling. This adds to the local prothrombotic and proinflammatory activity. In vitro studies have also shown that Stx is capable of inducing complement activation and deposition on EC cells (243).

Stx exerts direct damage on podocytes and disturb the filtration of blood in glomeruli. This causes proteinuria as more protein is excreted with urine (243). It has also been indicated that podocytes are indirectly damaged by complement through Stx activation of the alternative complement pathway (111).

Less is known about the effects of Stx on the remaining renal cell types. It is likely that Stx mediated effects on extraglomerular tubular cells are central in the process leading to tubular cell damage. This may result in decreased urine production and electrolyte disturbances (243).

These features combined are histopathologically seen as microthrombi, extensive endothelial damage and occluded lumina in glomerular capillaries, and fibrin depositions, mesangial cell expansion and tubular cell apoptosis. The local ischemic effect may in severe cases lead to development of acute cortical necrosis (8;243).

2.5.6 Neurological involvement

Neurological manifestations are seen in a variable proportion of STEC-HUS patients. Little is known about the pathophysiological processes involved. Experimental rat studies have shown that Stx2 enhances the expression of Gb3 receptors in neurons located in the striatum, hippocampus, cortex and thalamus. This mediated direct cytotoxic effects, cell apoptosis and dendritic abnormalities. These studies suggest a direct role of Stx2 in triggering the neurological dysfunctions seen in some STEC-HUS patients (244;245).

2.5.7 The complement system

Studies have shown that Stx activate the complement system through the alternative pathway and binds factor H: This indirectly damages podocytes in renal glomeruli (111;112). Complement activation has also been demonstrated on platelet-monocyte complexes, other blood cells and blood cell particles in HUS patients (246). In vitro studies have shown that Stx may modulate complement regulatory proteins, which in turn leads to impaired regulation and increased activation of the complement cascade (247). This has implicated a new way of Stx-mediated injury in STEC-HUS, and more interestingly a role of the complement system in its pathogenesis.

2.6 Surveillance of HUS and STEC

STEC is under routine epidemiological surveillance in the EU and the results are published in summary reports on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks (10;14). The notification of STEC infections is mandatory in most membership states, and Switzerland, Iceland and Norway (14). In addition, five membership states (France, Belgium, Italy, Spain and Luxembourg) have notification based on a voluntary system. The surveillance systems have full national coverage in all countries, except Spain, Italy and France. Meanwhile, routine surveillance of HUS exists in some European countries (15-17;124), and in certain regions in the US and Canada (137;248).

Surveillance of STEC based solely on microbiological confirmation and notification will underestimate the true incidence (249). An infecton with STEC may not necessarily result in seeking medical assistance, especially in cases with mild and/or asymptomatic disease. This is further highlighted by a 2016 study from Japan reporting an incidence rate of STEC in asymptomatic adult carriers of 84.2 per 100 000 population (250). Furthemore, mild cases may not be subject to microbiological diagnostics upon seeking medical assistance. Even when cases are investigated, the results may be limited by variations in laboratory methods and routines (17;63;249). The challenges in STEC surveillance have led some countries to use surveillance of HUS to follow trends and identify outbreaks of STEC infection, based on the average probability of STEC to cause HUS (27;251). In France, STEC surveillance is based on the occurrence of pediatric HUS, while Italy has sentinel surveillance based on the national HUS registry (14;17;27).

2.7 D^+HUS and STEC: Situation in Norway

STEC infection and HUS have generally been considered rare conditions in Norway. However, public interest and awareness has increased notably in the last decade. The main reasons for this is the occurrence of the first fatal outbreak of STEC-HUS in 2006 and the media attention surrounding suspected and confirmed outbreaks in later years. Notification of verified D^+HUS and/or STEC to the Norwegian Surveillance System for Communicable Disease (MSIS) is to date nominative and mandatory.

2.7.1 STEC and HUS in Norway

Althugh STEC O157:H7 was first isolated and described in the US in 1982, it was not identified in Norway until 1992 (124;125). The most commonly notified serotypes of STEC in Norway are O157, O103, O26, O145 and O91; these constitute almost half of all cases notified to MSIS (124). Measures to improve this have been implemented, especially following the 2006 outbreak. A total of 104 cases of STEC infection were reported between 1995 and 2004. Nearly half were reported as contracted abroad. In comparison, 487 cases were reported between 2006 and 2013. Whether this is due to improved diagnostics or an increasing trend is unknown (124;252). In contrast, the neighboring country of Sweden had 2890 reported in the same period (2006-20013) (253).

The first national outbreak of STEC in Norway was reported in 1999, when similar strains of O157:H7 were isolated in four patients; none of these patients developed HUS (185). Since then, several outbreaks have been recorded, including four on a national scale (Table 2). The most prominent was the 2006 outbreak of STEC O103:H25, where 17 pediatric cases were identified. Ten children developed HUS, one of which with a fatal outcome, resulting in the first known outbreak-related death in Norway. The origin of the outbreak was traced to cured mutton sausages (124;163).

2.7.2 Notification of STEC and HUS in Norway

Verified STEC and D^+HUS cases are notified to MSIS. The nominative notification of *E. coli* enteritis in children was made mandatory in 1977 (13). This changed in 1989 to include cases of *E. coli* enteritis in all ages and, in 1995, STEC were made mandatory notifiable. The notification of HUS was only mandatory in microbiologically verified STEC-HUS cases until 2006. Following the official evaluation of the 2006 outbreak (163;254), the criteria were changed to include all diarrhea-associated cases of HUS in December of the same year. Notification criteria for STEC were updated at the same time to include cases where microbiological verification was done by identification of *stx1/2*, regardless of culture status. Accordingly, the current notification criteria of STEC to MSIS is a clinically compatible case that is epidemiologically linked or is laboratory confirmed by

a) isolation of STEC positive for stx1 or stx2 gene(s),

b) detection of stx1 or stx2 gene(s) without isolation of strain,

c) detection of Stx in faeces without isolation of strain or

d) detection of STEC-specific antibodies in a HUS case.

In absence of stx, a HUS patient with eae positive *E. coli* and a patient with eae positive *E. coli* with a known genotype that has previously been identified in a HUS

case is also notifiable to MSIS. The latter is notified by the National Reference Laboratory for Enteropathogenic Bacteria (NRL) as a probable case of STEC that has lost its toxin-producing ability (STEC-LST).

MSIS is run by the Norwegian Institute of Public Health (NIPH) who are responsible for "*monitoring infectious diseases in Norway and contributing to international surveillance*" according to the Infectious Disease Control Act. *E. coli*-associated enteritis and D^+HUS are both considered "group A diseases". They are notified to MSIS with full patient identity by both clinicians and medical microbiological laboratories upon verification. Health personnel are also required to notify the Municipal Medical Officer (MMO) immediately when a suspected case presents. The MMO in turn notifies the NIPH, who must be notified directly through the 24 hour Infectious Disease Control duty officer if the MMO is unavailable. An early warning notification is required to be "*sent immediately in such a way that the sender is assured that the recipient has received the notification*" (124;255). When transmission from food products and/or animals is suspected, the MMO is also required to notify the local Food Safety Authority and vice versa (255).

2.7.3 Investigation and follow-up of STEC and HUS cases in Norway

An investigation is usually initiated immediately upon verification if the infection is contracted domestically (256). This applies to both sporadic cases and upon suspicion of an emerging outbreak. Implemented control measures and the level of investigation depend on characteristics of the isolated bacterial strain, the clinical symptoms of the patient, risk category concerning potential for further spread and number of suspected/verified cases. Verified HUS cases always trigger the strictest category of control measures and suspected outbreaks in institutions such as kindergartens and nursery homes are considered high risk.

The MMO is responsible for the investigation and preventive measures in sporadic cases and local outbreaks. This warrants close collaboration with the District Offices of the Food Authorities. The NIPH coordinates national outbreak investigations. The Norwegian Food Safety Authority aids in identifying the source of the outbreak.

The socioeconomic burden, as well as the cost and the workload related to the existing control measures warranted a revision of existing guidelines, and the guidelines for follow-up and control of STEC cases were revised in 2016 (211;257;258). This was performed in light of the general improvement to available detection methods, a noted increase in notified STEC and review of scientific research on risk factors for HUS development. The current guidelines thus differentiate cases mainly based on Stx subtypes into high-virulent (referred to in guidelines as "HUS-associated") or low-virulent cases and the control measures are tailored to each group. These groups are defined as follows:

- High-risk STEC
    - A STEC strain isolated from an HUS patient
    - An isolate/strain with identified *stx2* subtype(s) *2a*, *2c* and/or *2d*
    - An isolate/strain with identified *stx1* subtype *1a* in a child below six years of age with bloody diarrhea
    - An STEC-LST

- Low-risk STEC
    - An isolate/strain with identified *stx2* subtype(s) other than *2a*, *2c* and/or *2d*
    - An isolate/strain with identified *stx1* subtype(s) other than *1a* or if only stx1 or stx1a is identified in a case with no bloody diarrhea and/or above 5 years of age

In cases where *stx2*, in any patient, or *stx1*, in a patient below five years of age with bloody diarrhea, is identified without subtype in a primary laboratory, control measures are temporarily initiated as for a high-risk case pending subtyping. High-risk STEC cases of high-risk groups (e.g. kindergarten children, food industry workers, health personnel) are generally required to undergo three negative control samples. High-virulent STEC cases of low-risk groups and low-virulent STEC cases are now not required to present negative control samples and may return to work, school etc. when asymptomatic. Low-risk STEC cases of high-risk groups may return when free of symptoms for 48 hours.

2.7.4 Laboratory detection of STEC infection in Norway

Medical microbiological (primary) laboratories across Norway are mainly located at or associated to local or regional hospitals and surrounding primary physician offices. They are autonomous in their choice of diagnostic methods and not required to report changes in methods to the NRL. All suspected or verified human STEC isolates are sent to the NRL at the NIPH for verification and further characterization.

2.7.4.1 Local medical microbiological laboratories

Local guidelines and available detection methods vary between laboratories. In 1999, most laboratories only had the means to detect non-SF O157 by culturing; some also had agglutination kits for prominent serogroups. This is reflected by the yearly rate of serogroups identified in the late nineties and early 2000s (55). The first Norwegian laboratory to introduce PCR for STEC did so in the mid-nineties, while the last introduced PCR as late as 2016. Verified or suspected STEC samples were sent to the

NIPH for verification and further characterization throughout the study years, but this has been limited by locally available detection techniques.

The national outbreak of STEC O103 in 2006 led to prominent changes in laboratory policies. The Norwegian Government appointed an independent committee to evaluate the outbreak and how it was handled. Their report (254) included an evaluation of available detection techniques in the eight laboratories involved in the outbreak. Four of these had what was considered "satisfactory detection methods for all types of STEC" through PCR or DNA-hybridization. A fifth utilized ELISA, but only applied this to samples that were already O157 positive or sorbitol negative prior to the outbreak. The methods of the three remaining laboratories were considered "insufficient for identification of most STEC". These focused on direct identification of O157 and/or sorbitol negative cultures. According to the committee, this may have contributed to that only two out of seven O103 cases were identified prior to distribution of an outbreak warning, while eight out of eleven were identified after the warning.

The latter three laboratories generally reflected the available detection methods on a national level at the time; before 2006, the main focus of Norwegian primary laboratories was STEC O157 detection (55). This was further commented in the outbreak report, which cited results from a NIPH report (not available) where an STEC O103 (*eae* and *stx1*) test culture was sent out to 25 microbiological laboratories in the wake of the outbreak (254). Only six participating laboratories cited positive results using genetic detection methods. A further two had negative or uncertain results using EIA/ELISA, while the remaining 17 reported negative test results.

In their recommendations, the committee noted that the methods used by primary microbiological laboratories for detection of STEC should be improved (254). They further specified that minimum requirements should be established for diagnostic methods and sample selection to include genetic detection of *stx*. This would include optimization of forwarding of samples to laboratories where this was available. Nationwide improvements were made in the following years to adhere to these recommendations. This included the implementation of techniques for detection of *stx*/Stx, such as PCR, in a majority of the primary laboratories. The nature of the outbreak also contributed to a shift in focus, with general improvement of non-O157 detection methods.

Historically, Norwegian primary laboratories have not routinely screened for STEC in stool samples. Most laboratories adhere to this targeted approach based on clinical features or when requested by clinicians. However, in recent years, some of the leading Norwegian laboratories have implemented "broad screening" techniques for detection of enteropathogens. Multiplex PCR panels for simultaneous screening of multiple pathogens in stool samples had been introduced in six primary laboratories by the end of 2017.

2.7.4.2 The National Reference Laboratory for Enteropathogenic Bacteria

The status as a national reference laboratory for enteropathogenic bacteria, including STEC, was allocated to the Department of Bacteriology at the NIPH upon implementation of national medical microbiological reference functions in 2005. This functioned as a formalization of the already established routine use of the department to verify locally identified or suspected enteropathogenic *E. coli*. Presented here are the methods applied by the NRL and, where applicable, how they have changed over time.

Isolates are cultured using selective and differential media and fermentation. Since before 1999, all presumptive STEC isolates received by the NRL have been serotyped by slide agglutination using polyvalent and monovalent antisera (Sifin) against a range of O-groups. Further serological methods were earlier applied to identify H-antigens. These were later supplied to molecular serotyping.

PCR was introduced in July 1999 and has since been applied in molecular serotyping and subtyping of all relevant isolates. Until 2001, a modified ELISA was used to ascertain production of Stx1 and Stx2. This was replaced by a multiplex PCR for *stx1* and *stx2* in 2001, and later the same year expanded to include a PCR for *eae*. From July 2005, this was changed to an in-house developed multiplex PCR. This was expanded to also include primers for additional virulence genes, such as the gene encoding hemolysin (*ehxA*). In later years, matrix assisted laser desorption/ionization time of flight mass spectrometry has also been applied in epidemiological assessment.

Further characterization of isolates has historically been performed through DNA-profiling by MLVA. In 2018, work began to implement WGS as the primary method. The change from previous methods is currently ongoing.

2.8 Non-diarrhea-associated HUS

Non-diarrhea-associated HUS (D^-HUS) comprises around 10 % of all HUS cases (5). These patients usually present without prodromal diarrhea according to the D^+/D^- classification. However, it is important to note that diarrhea related to the acute phase has been reported in up to one fourth of cases. This may typically occur in patients with underlying genetic defects, where an infection triggers HUS (8). STEC-HUS is often initially suspected in such cases and later refuted through an investigation confirming a non-STEC etiology. Increased knowledge and improved diagnostic options have led to new classifications based on verified cause and pathophysiology (6). This will be addressed further in the discussion. Different triggering factors are involved in D^-HUS compared to D^+HUS, but they share the distinct pathophysiological feature of TMA (259).

Notification of D^-HUS cases is not mandatory in Norway. The epidemiological aspects of this diverse condition were hence unknown prior to this study.

2.8.1 Streptococcus pneumoniae-associated HUS

The most common cause of D^-HUS is HUS associated with infection with *Streptococcus pneumoniae* (SP-HUS). SP-HUS account for around 40 % of D^-HUS cases (5). The condition is considered an increasing problem globally, but remains relatively rare in high-income countries (260;261). It has conversely been reported as the most common cause of HUS in some Asian countries (262).

SP-HUS is usually preceded by an invasive and severe pneumococcal infection. This may be manifested as meningitis, pneumonia and/or empyema with septicemia (263;264). CNS involvement is often reported. SP-HUS patients require dialysis more often and are hospitalized longer than D^+HUS patients. Unlike in STEC-HUS, the use of antibiotics is indicated. This is especially important in cases with severe systemic infection. The short term prognosis is poor with mortality in the acute phase up to 25 %. The long-term renal prognosis is generally considered good compared to other types of HUS (5;265). Comorbidity and case fatality rate depend on the site of infection. They are higher in patients with pneumococcal meningitis complicated by HUS (263).

The understanding of the pathophysiology of SP-HUS has changed in recent years. It was long since discovered that a neuraminidase produced by the bacteria cleaves *N*-acetylneuraminic acid from glycoproteins on RBC, platelet and glomerular EC cell membranes. The result is exposure of the so-called Thomsen-Friedenreich antigen (“T-antigen"). This antigen may react with human plasma anti-T antibodies and cause cell damage (266;267). This was until recently thought to explain the SP-HUS pathophysiology. Studies have indicated that complement dysregulation and mass consumption related to mutations in complement genes may also play an important role (268).

2.8.2 Other causes of D^-HUS

In the classification applied in our work, the remaining causes of D^-HUS belong under the term atypical HUS (aHUS). This term covers several rare causes and/or triggers of HUS. The more recent classification separate these etiological entities into more specific categories (6). To elaborate on each of the numerous causes in this group is beyond the scope of this thesis. The most prominent subgroup of aHUS will be presented and a brief overview of additional causes provided.

2.8.2.1 Atypical HUS

The most prominent subgroup of aHUS was previously termed genetic HUS (269). Around 60 % of aHUS cases are related to either gain or loss of function or polymorphisms in genes coding for alternative complement pathway regulatory proteins. These may be either familiar or sporadic and lead to complement dysregulation and continuous complement activation when triggered. Several variants have been identified, including factor H, factor I, membrane cofactor protein

(MCP/CD46), C3, factor B, C4b-binding protein, clusterin and thrombomodulin (269;270). Patients may also have combinations of these variants. The genetic penetrance is variable, but predominantly high in recessive mutations in genes encoding for factor H and MCP (269). HUS events are triggered by an infection in approximately 70 % of these cases, often of the upper airways. The gene most commonly associated with mutations is *CFH*, encoding for factor H. These mutations are associated with the highest variety of outcomes (8;259).

Atypical HUS associated to complement dysregulation may also develop due to the creation of antibodies to factor H. It has also been shown that mutations in the so-called *DGKE* gene can cause HUS (271). *DGKE* encodes diacylglycerol kinase epsilon, an intracellular enzyme present in endothelium, platelets and podocytes. It functions by inhibiting a signal pathway that promotes thrombosis. The mutation results in a pro-trombotic state which in turn predispose for development of HUS. This knowledge has led to suggestions to change the classification of aHUS. The most prominent group is now classified as "complement dysregulation-associated aHUS", or simply "complement-HUS". It is still debated whether or not this should be used interchangeably with "aHUS" (200;269). In this paper, the term "genetic HUS" was kept in accordance with the papers presented.

2.8.2.2 Additional causes and associations in D$^-$HUS

D$^-$HUS may be idiopathic or associated with a wide range of additional causes (Table 4). However, there is invariably an identifiable genetic predisposition in such cases. It may often be unclear whether their association to HUS is strictly etiological or function as a triggering factor for underlying genetic abnormalities (6;259).

Table 4: Verified and associated etiologies identified in D$^-$HUS

Subgroup:	Specified:	References:
Genetic disorders of complement regulation	Mutations in CFH, CFI, MCP, C3, CFB, THBD	(6;200)
Acquired disorders of complement regulation	Anti-CFH antibodies	(6;200)
Other genetic disorders	DGKE mutation	(271)
Metabolic	Cobalamine deficiency, methyl malonic aciduria	(6;200)
Drug-induced	Quinine, oral contraceptives, calcineurin inhibitors, sirolimus, anti-VEGF agents	(6;200)
Non-STEC/Shigella dysenteriae I bacterial infections	*Streptococcus pneumoniae, Citrobacter freundii*[a]*, Pseudomonas aeruginosa,*	(6;48;272)
Viral infections	HIV, influenza A / H1N1	(6;200)
Parasitic infections	Cryptosporidium[a]	(49)
Cancer and cancer treatment	Chemotherapy	(6;200)
Transplantation-related	Solid organ transplantation, bone marrow transplantation	(6;200)
Pregnancy and pregnancy-related disorders	HELLP syndrome	(6;200)
Systemic disorders	Systemic lupus erythematosus, scleroderma, anti-phospholipid antibody disease, dermatomyositis	(6;200)
Glomerular diseases	Acute glomerulonephritis, post-streptococcal glomerular nephritis	(6;273)
Other rare causes		(6;200)
Idiopathic		(6;200)

[a] Presented with prodromal diarrhea and could also be considered D$^+$HUS

2.8.2.3 Clinical features and treatment of atypical HUS

Atypical HUS cases are often recurring and initially occur in childhood, especially before six months of age. They may present with variables degrees of thrombocytopenia and renal failure (269;274). Complications are more frequently seen and patients require dialysis more often and for a longer duration than in D$^+$HUS. The occurrence of severe organ failure and death is unpredictable. Around 50 % of pediatric patients progressed to ESRD or died 3-5 years after onset before the introduction of eculizumab (11;259). The prognosis depends largely on the cause of HUS and varies according to genetic factors involved (274).

A comprehensive international consensus approach to the management of atypical HUS in children was recently published by Loirat et al (200). This

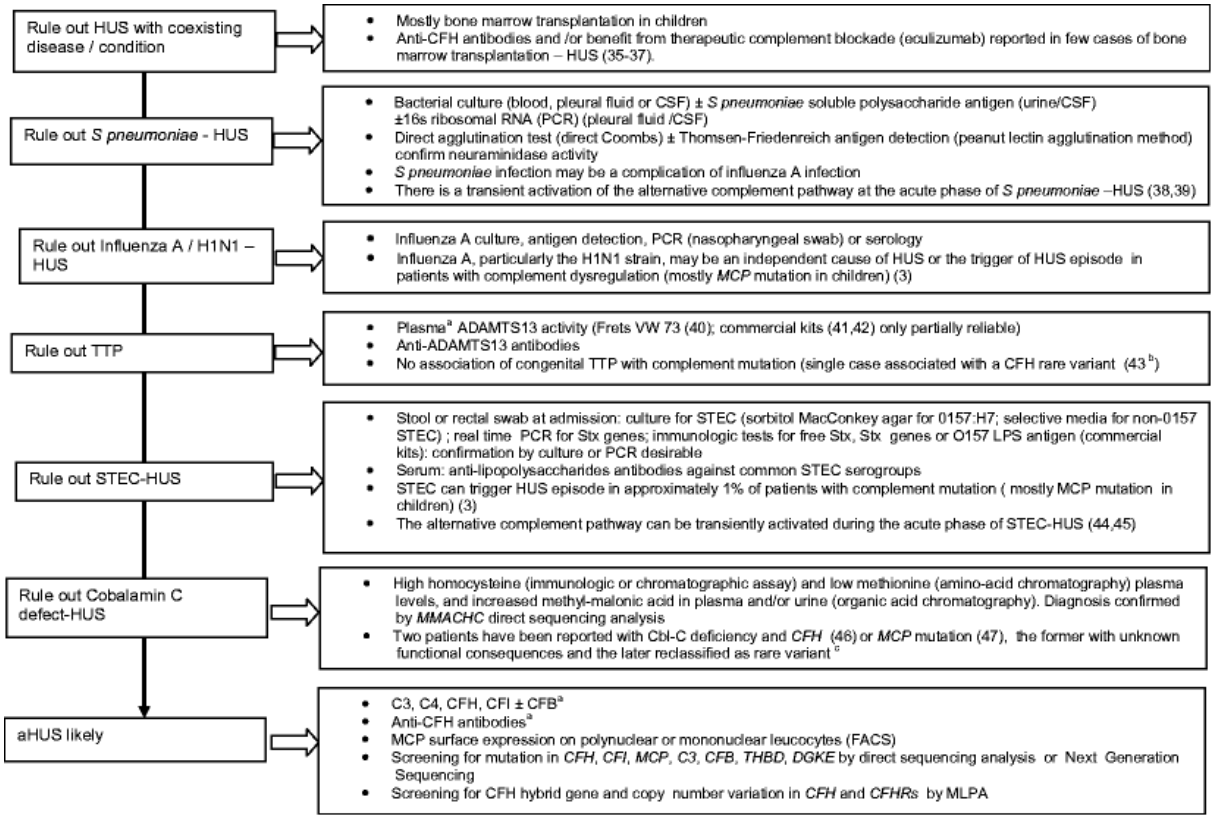
recommends different strategies according to disease progression and diagnostic possibilities. A detailed description of these strategies is beyond the scope of this thesis, although certain key points should be noted from the consensus approach:

- Effect and choice of therapy vary according to etiology.
- Some atypical HUS forms respond well to plasma exchange. , This especially applies to children with CHF mutations. However, the complication rate with plasma exchange is relatively high.
- AHUS patients generally have less successful results of kidney transplantation than D^+HUS patients. Complete genetic screening and anti-CHF antibody assay are necessary before considering transplantation. Prophylactic eculizumab treatment should be considered in patients with high risk of HUS recurrence.
- Eculizumab treatment has proved effective in aHUS and shown promise in treating and preventing post-transplant recurrence. Trials have shown effect in inhibiting the complement-mediated TMA activity and significant time-dependent improvement of the estimated glomerular filtration rate (eGFR) in affected patients (11). The risk of meningococcal infection is greatly increased in patients treated with Eculizumab and prevention by vaccination or prophylactic antibiotic treatment is crucial. First-line treatment with a clinical diagnosis of aHUS is eculizumab, preferably within 24-48 hours of onset or admission. Plasma exchange therapy or alternatively plasma infusion treatment should be initiated if eculizumab is unavailable.

2.8.3 D^-HUS diagnostic process

In their consensus approach, Loirat et al present a diagnostic algorithm for suspected aHUS in children (200). This comprehensive algorithm is shown in Figure 1. The initial step is to exclude STEC-HUS by stool cultures and PCR or immunologic assay for Stx in feces. SP-HUS is usually excluded by bacteriological cultures from body fluids and a negative direct Coombs test. Severely deficient ADAMTS13-activity in plasma indicates a TTP diagnosis and can be measured within a few hours using commercial kits. A thorough investigation should be initiated if these steps fail to provide a diagnosis. This includes an analysis of serum complement levels (C3, C4, CFH), genetic analysis for known mutations and polymorphisms and screening for CFH-antibodies. A low C3 level can indicate a complement defect. An early diagnosis is important because of the benefits of early treatment with plasma exchange and eculizumab. Family history, presence of probable triggering factors, age of onset, clinical context and presentation of symptoms is important information in the diagnosing process (259).

Figure 1: Diagnostic algorithm when aHUS is suspected (Loirat et al) (200)



2.9 Acute kidney injury

Acute kidney injury (AKI) is one of the three defining clinical features of HUS. AKI as a clinical entity is a major contributor to morbidity and mortality in critically ill patients (275-277). It may be defined as an acute decline in renal function, with falling glomerular filtration rate (GFR), and an inability to properly regulate the acid and electrolyte balance and excrete fluids and waste products (276;278), AKI can result from a host of different etiologies. There are various degrees of severity, ranging from mild temporary functional decline to end-stage renal disease (ESRD) (275-277). AKI is usually divided into three categories; prerenal, intrinsic/renal and post-renal. These reflect the underlying pathophysiological mechanism leading to the decline in renal function. The reported incidence of AKI varies greatly between countries and centers. It is generally considered to be increasing world-wide (276;278-280), and national cost estimates attribute up to 10 % of the health service expenditure to the condition and its associated complications (281).

2.9.1 Definition and classification

A wide variety of definitions and classifications have historically been used for AKI (278;282). The need for a more unison approach led to the proposal of the RIFLE (risk, injury, failure, loss, ESRD) classification in critically ill adult patients by the Acute Dialysis Initiative in 2004 (283). This utilized sCr, GFR and urine output to assess three grades of severity (risk of acute kidney injury, kidney injury and failure of renal function), in which the most severe measure should be used. Two additional levels were outcome classes (persistent loss of kidney function >4 weeks, persistent loss of kidney function >3 months), where the latter equaled ESRD. The Acute Kidney Injury Network (AKIN) modified them in 2007 (284), omitting the eGFR criteria. They also adjusted criteria to reflect the clinical impact of small changes in sCr and time as a variable in urinary output. Akcan-Arikan *et al* proposed the pediatric RIFLE (pRIFLE) criteria the same year (275). These were based on the RIFLE criteria, but implemented a change in estimated creatinine clearance (eCCl) based on the formula created by Schwartz (285). The Kidney Disease: Improving Global Outcomes (KDIGO) group later published the KDIGO Clinical Practice Guideline for AKI in 2012 (286). This attempted to merge RIFLE, AKIN and pRIFLE to create the KDIGO criteria, where AKI is defined by the presence of one of the following:

- Increase in sCr by ≥0.3 mg/dL (≥26,5 μm/L) within 48 hours
- Increase in sCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days
- Urine volume <0.5 mL/kg/hour for 6 hours

The RIFLE criteria utilize an estimated baseline sCr based on the Modification of Diet in Renal Disease (MDRD) formula (287) and an assumed baseline eGFR where a reliable baseline sCr cannot be obtained (283). The AKIN criteria made this unnecessary by relying on two sCr values taken within 48 hours (284), while the concept of estimated baseline sCr was retained in the KDIGO criteria (286).

There has been some debate surrounding the KDIGO criteria, particularly concerning its applicability in local clinical practice (288;289). One of the main arguments was that the definition and classification criteria were largely ungraded. Several studies have since shown their strength in identifying AKI and prediction of mortality in critically ill patients (290-292). A modified classification for use in neonates has also been suggested (293). The KDIGO criteria remain one of the most widely accepted definitions to date in clinical research (276;282). Despite this, the definitions are still inconsistently used, both modified and in their original form (281;282;294). They also provide different estimates of occurrence, often depending on local conditions and patient characteristics (291;295), This shows that there is still work needed towards creating a single, universal definition of AKI.

The introduction of consensus based definitions was paralleled by a shift in terminology from acute renal failure to AKI (283;286). This was necessary to encompass the broad spectrum of the condition, from small alterations in kidney function to acute need of RRT.

2.9.2 Epidemiology

An estimated 13.3 million people world-wide are affected by AKI every year, of which 85 % live in developing countries (296). More than one fifth of hospitalized patients are affected, and proportions ranging from 5.2 % to 67.2 % have been reported in the critically ill (296;297). AKI is considered more common in adults than in children and the reported incidence increases with age (280;298). Studies also show a predominance of male patients in AKI cohorts (299).

The myriad of AKI definitions and criteria has been a major challenge in epidemiological research (278;282;286;295). This especially applies to community-acquired AKI (CA-AKI) (300). Studies are usually concentrated to specific centers or regions and based on surveillance networks and registries (277;301;302). These often focus on in-hospital populations, hospital-acquired AKI (HA-AKI), in specific patient groups. National and nationwide epidemiology reports are scarce. A recent national International Statistical Classification of Diseases and Healh Related Problems 10th Revision (ICD-10) code based study from England estimated a total non-dialysis requiring AKI incidence of 3995 per million hospitalized people, of which 63.4 % were aged above 75 years (303).

Current literature indicate a general increase in AKI incidence world-wide (297;303-305), Several explanations have been suggested to account for this, such as application of more sensitive diagnostic markers and definition criteria, development

and use of (nephrotoxic) medications, the availability of dialysis and an aging population (300;303;306). Available studies on AKI epidemiology are also disproportionately distributed. The majority stem from developed, high-income countries, which likely contributes to an underestimation of incidence in developing countries (300). Conversely, some reports suggest the variation may be due in part to the methodological heterogeneity seen in epidemiologic AKI studies (307).

Epidemiological reports on the underlying etiologies of AKI vary between different countries and centers (297;304). This is influenced by conditions ranging from climate to socioeconomic status, demography and availability of health care (280;281;297;304).

2.9.2.1 Adult AKI epidemiology

A world-wide meta-analysis by Susantitaphong *et al* found that approximately one in five adults experience AKI during hospitalization (300). The analysis included various in-hospital cohorts, including intensive care patients. This estimate was similar to that found in a single center study including nearly 20 000 hospitalizations (308). However, the proportion has been shown considerably lower in other studies focused on overall hospital admissions (309;310).

Several studies have presented estimates on adult AKI incidence. In a regional population-based cohort from the United Kingdom, Sawhney et al found an adjusted adult incidence rate of approximately 150 AKI episodes per 10 000 population over a ten-year time span (307). A 2015 nationwide, cross-sectional survey from China found that AKI affected around 1-2 % of all adult patients admitted to hospital, thus an estimated 2.4-3.1 million Chinese adults per year (281). A large prospective study including all tertiary centers in the Madrid area found that AKI affected 1.5 patients per 1000 adults (> 14 years of age) admitted and a community incidence of 209 cases per million population (311). In an insurance based US study, Hsu *et al* reported a peak in community-based non-dialysis requiring AKI incidence rate of 522.4 per 100 000 person-years in 2002-2003 (298). A later US report showed a rate increasing from 263.5 (age group 18-44) to 10 449.9 (age group 85+) inpatient stays per 100 000 population with an all-listed AKI diagnosis in 2014 (280), The proportion of CA-AKI in cohorts is reportedly 50 % or more, usually higher in developing countries (281;309;311-313).

The 2015 AKI-EPI study covering ICU patients in 97 centers world-wide found that the most common causes were sepsis, hypovolemia, drug related and cardiogenic shock (314). In US studies, AKI is most frequently seen in cases of sepsis, surgery, congestive heart disease and respiratory conditions (280). Reports from Africa and Asia point to prerenal conditions such as sepsis, hypovolemia, toxin exposure and surgical procedures as major causes (281;309;310;315). The 2015 Chinese study also found that nephrotoxic drugs were implicated in more than 70 % of cases, although direct causality was often difficult to determine due to comorbidities (281).

2.9.2.1 Pediatric AKI (pAKI) epidemiology

In their meta-analysis, Susantiaphong and colleagues also estimated than as much as one third of hospitalized children are affected by AKI (300). Reports depicting pediatric patients from noncritical admissions have shown lower numbers, down to 5 % (316). Conversely, AKI has been shown to affect more than 50 % of patients in some high risk patient cohorts (317).

A limited number of studies have estimated incidence from pAKI cohorts. A nationwide US cross-sectional analysis of 2009 data reported a rate of 3.9 cases per 1000 pediatric admissions (318). Another US report presented a rate of inpatient stays with AKI at 22.1 per 100 000 population in children aged up to and including 17 years (280). A UK tertiary care center study found a yearly incidence of 0.8 children per 100 000 total population between 1984-1991 (319). Another tertiary center study from Thailand spanning 22 years found a peak incidence of 9.9 cases per 1000 pediatric patients (320).

Reported pAKI etiology varies both between developed and developing countries, but also between national centers (276;304). HUS is still considered the most common cause of pAKI in Europe (321). This is reflected by some studies (319;322), while others show a different distribution (301;302). The arguably most robust studies on pAKI etiology in developed countries stem from North America. These found that pAKI is most commonly seen in relation to cardiac surgery, sepsis and nephrotoxin exposure (323). Smaller, center-based studies have from the same region have also implicated HUS and oncologic pathologies (324).

Pediatric AKI in developing regions in Africa and Asia has largely been dominated by prerenal causes (320;325-328). This mainly involves dehydration related to gastroenteritis. However, improving living conditions means that the pattern might be changing (325;326). Other commonly reported causes of pAKI in developing countries include sepsis, HUS, glomerulonephritis and toxin exposure (320;327-331).

2.9.3 Pathophysiology

AKI is usually considered a multifactorial condition and can follow numerous different etiologies. These etiologies can be divided into pre-renal, renal (intrinsic) and post-renal, although clinical phenotype varies according to definition applied (332). However, certain common pathophysiological processes are thought to occur sequentially and contribute to AKI development (333). Current established knowledge of AKI pathophysiology is limited as understanding of mechanisms and existing models are often derived from animal models, observational studies, or from specific disease groups that make extrapolation difficult (334). The key concepts are briefly summarized in this section as most aspects overlap with those covered in HUS.

2.9.3.1 Common pathophysiological processes in AKI

Hemodynamic instability with relative or persistent hypotension is considered an important factor and has been associated with the development of AKI in clinical and surgical settings (333). However, several studies have shown that AKI may develop in absence of reduced renal blood flow (335). This has led to focus on the role of the intrarenal microcirculation as a crucial component in AKI development.

Glomerular blood flow is inhibited partly due to vasoconstriction brought on by an increase of vasoconstrictive factors and sympathetic activation (332). Reduced glomerular blood flow disturbs perfusion of both the glomeruli and peritubular microcirculatory networks (336). Endothelial dysfunction leads to increased capillary permeability with interstitial edema can further disrupt microcirculatory flow and hamper tissue oxygen diffusion (333). These changes may occur as focal perfusion deficits, even in a state of preserved overall renal blood blow (336).

Endothelial activation and dysfunction may result from exposure to inflammatory mediators and vasoconstrictive factors (332;333). Activation promotes increased adhesion and migration of leukocytes. Reduced oxygen availability and leukocyte adhesion may in turn damage tubular cells (332;337). Tubular cells may also be damaged by exposure to other filtrate substances and directly impact on GFR through triggering of the tubuloglomerular feedback mechanism (333). Both endothelial and tubular cells further release pro-inflammatory cytokines that further triggers inflammation (333;337). Inflammation may also follow glomerular deposition of immune complexes in auto-immune conditions. These inflammatory processes contribute to perturbed blood flow and degradation of natural anticoagulants. This combines with endothelial damage mediating coagulation to induce formation of microvascular thrombi and capillary plugging (333).

Tubular cell damage thus drives the pathophysiological progress (332). Further damage may eventually lead to tubular cell layer collapse and loss of basement membrane function. The ensuing mixed debris form obstructive cylinders and increase intratubular pressure, which in turn lead to decreased GFR.

The role of venous congestion in AKI stems from conditions that lead to elevated central venous pressure, such as congestive heart failure (333). Increased backward pressure may lead to tubule compression and falling glomerular pressure gradient. Tubular obstruction may conversely result from obstruction in the lower levels down to the urethra (333).Progression from AKI to CKD and ESRD in not fully understood, but is thought to involve development of tubulointerstitial fibrosis due to nephron loss, glomerular hypertrophy and endothelial injury with decreased vascular supply (338). AKI also has a role in so-called organ crosstalk (339). Research show that renal pathophysiological processes involved in AKI are directly involved in concurrent organ dysfunction in other organs, such as the lung, brain and heart. Further exploration on these themes is considered beyond the scope of this thesis.

2.9.3.2 Etiological distribution of AKI

Pre-renal AKI is considered the most common etiological group (332). The common defining feature is decrease of renal perfusion leading to reduced GFR, mainly due to hemodynamic instability. This may result from hypovolemia caused by excessive fluid loss or insufficient intake, pump defect or excessive circulatory vasodilation. These are usually reversible with early hemodynamic stabilization, in which case renal tissue damage may be avoided (278;332).

The challenge with this distribution is that prolonged pre-renal may result in intrinsic AKI (278). There is thus an etiological cross-over between the two groups. Other intrinsic causes are primary glomerular, interstitial or vascular renal diseases and conditions leading to renal ischemia (332). These conditions directly affect the renal parenchyma. Finally, post-renal AKI constitutes causes that obstruct the urinary tract on any level below the renal tubules (332).

2.9.4 Risk factors for AKI development

Various risk factors for development of AKI have been identified. Older age is the major demographic risk factor (324;340). Clinical risk factors include hypoxemia, hypotension, congestive heart failure, sepsis, volume depletion, CKD, nephrotoxin exposure, neurologic dysfunction and diabetes (324;340). In addition, certain biochemical risk factors have been demonstrated, such as specific interleukins and plasminogen activator inhibitors (340). Notably, some risk factors defined by age-related comorbid conditions are naturally more common in adults than in children (340).

2.9.5 Diagnosis of AKI

A typical diagnosis of AKI is mainly based on accumulated nitrogen metabolism end products (sCr, plasma urea) and/or decreased urine output (283;334). Further assessment includes other biomarkers, blood work-up, GFR, urinalyses, such as urine microscopy, measuring electrolytes, sodium and urea excretion and sediment analysis, and renal ultrasonography (334;340). These measures may contribute towards identifying an underlying cause or trigger and establish the mechanism of injury as prerenal, intrinsic or post-renal (334). This also warrants consideration of epidemiological and clinical setting, risk factors and clinical features. More specific blood work (such as antibodies) and diagnostic procedures are applied relative to the suspected diagnosis. A renal biopsy may be performed where clinically relevant for further treatment. In recent years, utilization of information technology in electronic health systems to facilitate in-hospital AKI detection has also shown promising results (341).

2.9.5.1 AKI biomarkers

SCr has historically been the preferred biomarker in AKI assessment (338). This is partly because of its availability and low associated cost (282). It remains widely used to date despite well-known limitations. SCr may be influenced by a host of factors, including age, weight, sex, diet, fluid administration, drugs and muscle mass (276;334;338). SCr levels are usually not notably elevated until 24-72 hours following renal damage. It generally remains normal until around a 50 % loss of nephrons or GFR decrease below 60 mL/minute/1.73 m^2 (282). Active tubular creatinine secretion also leads to an unpredictable overestimation of GFR that may be further influenced by secretion inhibiting drugs (282). Thus, sCr levels are variable in reflecting real-time renal function.

The limitation of sCr has led to extensive investigations for more suitable biomarkers for (early) AKI diagnostics (334;338;342). The advantage compared to sCr is earlier detection and localization of injury that allow a more thorough assessment of treatment effect and prognosis (342). Arguably, the most prominent of the novel biomarkers are cystatin C and neutrophil gelatinase-associated lipocain. Both show an earlier rise in serum concentration than sCr. The former is also potentially a more stable predictor of GFR as it is less affected by muscle mass (343). However, despite their potential for early AKI detection compared to sCr, studies have yet to show any significant advantage of novel biomarkers on outcome (282).

2.9.5.2 Staging of AKI

The KDIGO criteria include three staging grades for AKI severity (286), that have been validated in later studies (292). This is based on sCr and urine output, except for a stage 3 criteria of RRT initiation or an absolute eGFR threshold for pediatric patients. Baseline sCr is based on MDRD (287) if unavailable, as for definition criteria. Patients are staged according to the set of criteria that give them the highest (worst) stage as follows (286):

Stage	**sCr**	**Urine output**
1	1.5-1.9 times baseline, OR Increase by ≥0.3 mg/dL (≥26,5 µm/L)	<0.5 ml/kg/hour for 6-12 hours
2	2.0-2.9 times baseline	<0.5 ml/kg/hour for ≥12 hours
3	Increase to ≥4.0 mg/dl (≥353.6 µm/L), OR RRT initiation, OR eGFR decrease <35 ml/min per 1.73 m^2 if below 18 years of age	<0.3 ml/kg/hour for ≥24 hours, OR Anuria for ≥12 hours

2.9.6 Treatment of AKI

In 2015, a Commission established through collaboration between the International Society of Nephrology and *the Lancet* published results from their 0by25 initiative (297). The aim of this initiative was zero preventable AKI deaths by year 2025. However, no pharmacological treatment options have been proven effective in AKI (334;338). Management of underlying conditions and appropriate supportive therapy through adjusted fluid administration and RRT are the hallmarks of AKI treatment.

2.9.6.1 Prevention

Prevention of AKI remains a fundamental approach in the absence of specific treatment options. Close hemodynamic monitoring and resuscitation are essential, especially if predisposing prerenal factors are present in critically ill patients (334). Recognition and adjustment of nephrotoxic drugs is also vital, as these contribute to AKI in a large proportion of patients (281;334;338).

The lack of effective therapeutic measures for existing AKI and predictive inadequacy of disease severity scoring systems prompted introduction of the renal angina concept (340;344). This combines assessment of known risk factors with early diagnostic signs to predict the development of AKI in at-risk patients. It utilizes threshold changes in sCr and duration of oliguria in adults, whereas fluid overload and eCCl decrease is applied in children. The concept was further developed into the renal angina index, which has been validated for risk-stratification in critically ill patients (345;346). Identification of at-risk patients using the index potentially provides a more targeted approach using biomarkers to predict development of AKI (344-346).

2.9.6.2 Acute phase treatment

Acute phase AKI treatment is a continuation of preventive and supportive measures; target the cause while maintaining fluid balance (276;334;338). This includes symptomatic treatment of complications, such as hyperkalemia, metabolic acidosis and fluid overload. Some underlying causes dictate additional therapeutic measures. Examples of this are aggressive fluid resuscitation and urine alkalinisation in rhabdomyolysis-associated AKI, and albumin administration and vasopressin in hepatorenal syndrome (334). Patients who develop severe AKI may ultimately need RRT (338). Indication for initiation of RRT may vary locally, but common criteria are severe oligoanuria, critical hyperkalemia or metabolic acidosis, fluid overload, substantial azotemia or clinical uremia (334).

Some central aspects surrounding RRT treatment are subject to conflicting study results. High-dose RRT has been widely used since it was shown to reduce mortality, but substantial RCTs have since been unable to confirm this versus lower doses (334;338). Continuous RRT is usually the preferred mode in unstable patients, while study results have yet to show convincing differences over intermittent therapy (334;347). Hemodialysis is often reported as the preferred modality, but systematic

reviews have not shown superiority compared to hemofiltration or peritoneal dialysis (348;349). Furthermore, no conclusive evidence currently exist on whether early versus late RRT has significant impact on AKI outcome (338), or the ideal timing of discontinuation of RRT (334).

2.9.6.3 Long-term treatment and follow-up

Increasing evidence links AKI episodes with long-term renal sequelae (334;350). It is therefore recommended that at-risk patients receive proper follow-up care after discharge. This should ideally include continuous assessment of renal function biomarkers, blood pressure monitoring and urinalysis to screen for persistent proteinuria and hematuria (350).

2.9.7 Prognosis of AKI

The reported short- and long term outcome of patients affected by AKI varies with factors such as patient demographics, etiology and severity of injury (278;351). A meta-analysis found a pooled incidence of CKD of 25.8/100 and ESRD of 8.6/100 patient-years in previous AKI patients (352). The systematic review by Susantitaphong et al estimated an overall mortality of 23 % in AKI patients; 23.9 % in adults and 13.8 % in children (300). Reported AKI mortality in critically ill patients range from 17.1 % to 64.7 % (338).

2.9.7.1 Prognostic factors in AKI

Prognostic factors that predict a worse outcome in AKI patients include oligoanuria, hypervolemia, abnormalities in urine sediment and severity of renal dysfunction (310;353;354). In the short term, AKI is associated with prolonged hospitalization, need for mechanical ventilation and increased costs across several conditions (318;354). Studies also show an association between AKI and long-term renal sequelae, such as persistent proteinuria and hypertension, CKD and ESRD (278;350-352). The risk of CKD increases with AKI severity, older age, female sex, albuminuria and a high baseline sCr (351). This in turn potentially connects acute episodes to the considerable multifaceted morbidity of chronic renal disease (350). AKI also accelerate progression to ESRD in CKD patients (338). AKI is furthermore associated with an elevated long-term risk of cardiovascular events (355).

2.9.7.2 Mortality in AKI

AKI is considered to cause or contribute to around 1.7 million deaths annually across the globe (296). It is associated with increased mortality, also when adjusting for presence and severity of comorbid conditions in hospitalized patients (308;324;354;356). This applies to both in-hospital and long term mortality (357) and increases with the severity and duration of AKI (300;314;354;355). Even small increases in sCr show a 4-fold greater in-hospital mortality compared to no increase (354). Other factors associated with increased in-hospital mortality in AKI patients is

delayed recognition, lack of renal referral and need for mechanical ventilation (281;310). Mortality is greater in HA-AKI compared to CA-AKI (312). In addition, AKI is independently associated with an elevated risk of long-term cardiovascular mortality (355).

3. Aims of the thesis

The main aim of this thesis was to:

- Examine and describe the central aspects of hemolytic-uremic syndrome (HUS) in children in Norway to determine the extent and burden of the condition on a national basis

This was assessed through the following aims, listed according to the paper in which they were addressed:

- **Paper I**
  a) Describe the yearly and age-specific occurrence and incidence of HUS in children in Norway in the period from 1999 up to and including 2008
  b) Describe the distribution of etiologies of HUS in children in Norway, and specifically to determine the proportion of diarrhea-associated HUS (D^+HUS) cases with verified Shiga toxin-producing *Escherichia coli* (STEC) infection
  c) Evaluate whether there is an underreporting and/or underestimation of HUS and/or STEC based on extrapolation of the number of cases identified in the study, by comparing to the number of cases notified to the Norwegian Surveillance System for Communicable Disease (MSIS)

- **Paper II**
  d) Investigate the observed increase of notified STEC cases in Norway from 2007-2017 in order to assess the effect of broad screening PCR implementation at the medical microbiological laboratories on the distribution and characteristics of notified STEC cases

- **Paper III**
  e) Describe the clinical features, applied theurapeutic interventions and long-term complications in cases of HUS in children in Norway

- **Paper IV**
  f) Estimate the yearly and age-specific occurrence and incidence of acute kidney injury (AKI) in children in Norway in the period from 1999 up to and including 2008
  g) Describe the distribution of etiologies of AKI in children in Norway, and specifically to determine the proportion of HUS cases to assess the burden of HUS in AKI cases in Norway

4. Materials and methods

4.1 Study design and data collection

4.1.1 Papers I, III and IV

Papers I, III and IV were collectively performed as a retrospective, descriptive study. Data were collected directly from medical records of relevant patients below 16 years of age that were admitted to hospital in Norway from January 1, 1999, to December 31, 2008. In HUS cases, these data included demographics (age, municipality, gender), clinical information (underlying/former disease(s), hospital of admittance, date(s) of admittance(s), readmittance and follow-up, diagnose codes, medical procedures, medications, complications) and laboratory information (test values, methods, results). In AKI and nephritis cases, these data included age in full years, gender, hospital of admittance, year of admittance, diagnose codes and etiology. The highest identifiable creatinine value was collected in nephritis cases.

Medical records were assessed in electronic and/or printed form, according to local availability. This varied as several hospitals included had implemented a fully electronic medical record system during the study period. Included data variables were predetermined through a pilot project to assess their availability in standard medical records.

Potential cases were identified by performing local medical record searches for patients with International Statistical Classification of Diseases and Healh Related Problems 10th Revision (ICD-10) codes D59.3 (HUS), N17 (AKI) and/or N00/N01/N05 (acute nephritic syndrome / rapidly progressing nephritic syndrome / unspecified nephritic syndrome). Nephritis cases were included to identify potentially misdiagnosed cases of HUS and/or AKI. The additional ICD-10 codes included were decided through consulting our attached expert on pediatric nephrology.

Forms made in EpiData (358) were used to register data. The data were collected at all pediatric departments in Norway that had confirmed cases of HUS and/or AKI in the study period, and that had not immediately transferred them to secondary/tertiary hospitals. We initially assessed all hospitals with pediatric care capacity and identified those that did not require us to collect data on-site. A total of 37 hospitals (and health centers) were identified as having some type of pediatric care capacity by either available guidelines and/or through direct contact with local health personnel. We excluded 19 sites from visitation as they would immediately transfer any relevant pediatric patients. Six of these had HUS and/or AKI cases admitted before transfer or during follow up. In these cases, we either relied on the presence of medical records transferred with the patient or requested the missing information by mail. The remaining 18 hospitals all confirmed the admittance of HUS and/or AKI

cases in the study period. One was not directly visited as it had a shared electronic journal system with one of the other 17 hospitals.

In June and July of 2009, Jenssen and Hovland performed a nationwide tour to collect data from the more remote hospitals included. As medical students, planned trips to collect data from relevant hospitals were performed when possible. In the autumn of 2010, Jenssen applied and had the project accepted into the Medical Student Research Program (MSRP) at the Faculty of Medicine, Oslo, in January 2011. In the following months, Jenssen completed the data collection process through regular trips to the remaining hospitals. All data collected up until 2011 was done with both present, although varying between reviewing medical records together or separately according to local conditions. The only exception was at two hospitals on the nationwide tour where timing and funds necessitated split routes. From initiation into the MSRP, Jenssen collected the remaining data alone. Our attached experts on pediatric nephrology were available for phone consultation in unclear and/or difficult cases during the entire data collecting process. The coordinating center of the study was the Norwegian Institute of Public Health.

Limited surveillance data on cases of HUS and STEC notified from the 1st of January, 1999, to the 31st of December, 2008, were exported from MSIS as Microsoft Excel spreadsheets. Information on microbiological findings was extracted from MSIS for the notified cases of STEC-HUS. This was possible as MSIS receives data on microbiological characteristics both from regional laboratories and the National Reference Laboratory for Enteropathogenic Bacteria (NRL) at the NIPH.

4.1.2 Paper II

Paper II was conducted as a retrospective quality control study. Data on all STEC cases identified and notified to MSIS from January 1, 2007, to December 31, 2017, were extracted, including demographics (age, sex, place), clinical presentation (symptoms, hospitalisation) and laboratory findings (date of sampling, diagnosing laboratory, serotype, *stx* subtype, presence of *eae* and *ehxA*, MLVA-type). The same data were also extracted on all HUS cases, specified in NIPH guidelines as acute renal failure and at least microangiopathic haemolytic anaemia and/or thrombocytopenia with an epidemiological link, notified to MSIS in the same study period. Incomplete laboratory data in MSIS was supplemented with data from the NRL registry where available. In addition, lmited data (date verified and notified) were also extracted on selected concomitant bacterial infections for all notified STEC cases from laboratories with broad screening PCR methodology by cross-checking MSIS registries.

These data stem from mandatory notification reports to MSIS, provided by both clinicians and laboratories upon verification of relevant conditions, and data from further analyses provided by the NRL. The NRL receives presumptive STEC isolates for verification and characterization from all the Norwegian medical microbiological laboratories. All concerned condiditons are considered “group A diseases”, and are

thus nominatively notified to MSIS, which enabled cross-checking of data. Further expansion of these routines was provided in the introduction of this thesis.

Furthermore, information concerning the implementation of broad screening PCR methodology at the medical microbiological laboratories was gathered from a national survey on laboratory practice from 2017, and through personal communication with the laboratories if necessary.

4.2 Case definitions

4.2.1 Papers I, III and IV

For inclusion in Papers I, III and IV, a case had to be below 16 years of age at hospital admittance and be admitted to a Norwegian hospital between 01.01.1999 and 31.12.2008.

A HUS case was defined as:

- a case clinically compatible with all the following laboratory findings of
    - thrombocytopenia (< 150 x 10^9/L), AND
    - anemia (hemoglobin level (Hgb) < 10.5 g/dL), with elevated serum lactate dehydrogenase (LD) (>500 U/L), AND
    - acutely reduced renal function, with sCr
        - > 35 µmol/L for patients < 1 years of age
        - > 80 µmol/L for patients 1-15 years of age

      AND
    - EITHER
        - reported presence of fragmented RBCs (schiztocytes) on peripheral blood smear; a sign of microangiopathic changes consistent with hemolysis and an important part of HUS pathophysiology (2)

          or
        - if peripheral blood smear was missing in the journal; probable clinical HUS confirmed by consulting a clinician with expertise in pediatric nephrology*

** The pediatric nephrologists were also consulted when other unclarities arose, such as in four cases with evident HUS that did not match creatinine criteria, discussed*

later. The criteria were intended to reflect this, but it was not specified in the definition criteria provided in Papers I, III and IV, and was thus not altered here.

A D^+HUS case was defined as a HUS case with EITHER:

- probable STEC-HUS, with a clinical presentation of either prodromal diarrhea without verifiable causative etiology, OR
- STEC-HUS, defined as a HUS case with laboratory verified STEC infection

A D^-HUS case was defined as any non-diarrhea-associated HUS case without verifiable STEC infection and/or HUS of verified non-STEC causality.

An AKI case was defined as a case with:

- a primary or secondary initial diagnose of AKI (N17), and/or HUS (D59.3) and/or acute nephritic syndrome/rapidly progressive nephritic syndrome/unspecified nephritic syndrome
  and
- a confirmed history with a sCr elevation of
  - > 35 µmol/L for patients < 1 years of age
  - > 80 µmol/L for patients 1-15 years of age

AKI cases related to birth asphyxia or post-kidney-transplantation acute graft failure were excluded. In AKI patients with multiple admittances and/or recurrence of AKI, only the initial occurrence was included.

4.2.2 Paper II

In accordance with the aim of the paper, all cases notified as STEC to MSIS were included in the study as individual STEC cases, regardless of whether they fulfilled the notification criteria at the time of notification. Furthermore, all cases notified as HUS to MSIS are registered as STEC. Accordingly, in the paper, HUS cases were treated as a subgroup of STEC cases with an outcome of HUS, regardless of whether the presence of STEC had been verified. This was not specified in the paper, but is provided here for clarity in the context of this thesis. The notification criteria for STEC and HUS were provided earlier in the thesis (see 2.7.2 and 4.1.2).

We further categorised STEC cases based on the 2016 revised guidelines (258).

A case was categorised as having a high-virulent STEC infection if

i) positive for stx2 subtypes 2a, 2c, 2d, or

ii) positive for stx1 subtype 1a in a patient ≤ 5 years with bloody diarrhoea, or

iii) notified as a HUS-patient, or

iv) negative for stx, but eae positive E. coli strain (STEC-LST) with a genotype (MLVA-type) previously seen in a HUS case

A case was categorised as having a low-virulent STEC infection if

i) positive for stx1 (not 1a in a patient ≤ 5 years with bloody diarrhoea), or

ii) positive for stx2 subtypes 2b, 2e, 2f, 2g

Cases that did not fulfil any of the above-mentioned criteria due to missing and/or insufficient data were categorised as having an unclassifiable STEC infection.

We defined a concomitant bacterial infection as notification of a pathogen included in the broad screening PCR panel (*Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia* spp., and/or other enteropathogenic *E. coli*) from the same laboratory and same sampling date as the STEC case.

4.3 Statistical analysis

In Papers I, III and IV, statistical calculations were performed using Microsoft Excel. These are presented as proportions, median and annual average values with ranges and as incidence rates. Estimated glomerular filtration rate (eGFR) was estimated using the height-independent Pottel eGFR equation (359).

In Paper II, all statistical analyses were performed in Stata version 14 (Stata Corporation, College Station, Texas, USA). Chi-squared test for categorical variables was used to examine the distribution of demographics (sex, age, seasonality, and place of infection), clinical (hospitalization) and microbiological (serogroups and virulence profile) characteristics between cases with high-virulent and low-virulent STEC infections. Wilcoxon's rank sum test was applied to examine the differences between the two groups with respect to continuous variables (age). Times series analysis were conducted allowing for trends and seasonality (1 year periodicity) and adjusted incidence rate ratios (aIRRs) with 95% confidence intervals (CIs) calculated using negative binomial regression on 2007-2017 data for cases reported from laboratories that implemented broad screening PCR and from laboratories that did not implement this screening method.

In all papers, incidences rates were calculated using population numbers acquired from official registries; Statistics Norway (Statistisk Sentralbyrå; www.ssb.no).

4.4 Ethical considerations

The study constituted by Papers I, III and IV was approved by the Regional Ethical Committee South East A (Regional Etisk Komite Sør-Øst A). Dispensation from patient confidentiality regulations requiring informed consent prior to accessing patient medical records was granted from the Norwegian Ministry of Health (Sosial- og Helsedirektoratet). This was necessary as potential cases would only be identifiable through the review of medical records. Once HUS cases were identified, the parents were notified and could elect to withdraw from the study. None chose this option. Data variables collected and presented in identified AKI and nephritis cases were limited. Notification of parents after identification was not required. Data files containing personally identifiable data were encrypted and stored according to the information security standards of the NIPH.

Due to the study design of Paper II, no approval or permission was required. The study was based on data from notifications to MSIS and bacterial isolates from the strain collection at the NRL. The Norwegian Communicable Disease Control Act and its companying regulations oblige the NIPH to perform national surveillance of communicable diseases, including STEC infections. In accordance with this, the present study and its potential findings were considered as assessment of the surveillance and guidelines provided by the NIPH. This qualifies as quality control of one of the imposed tasks of the NIPH. Accordingly, ethical approval from a Regional Ethical Committee was not required and informed consent was not required from the patients involved.

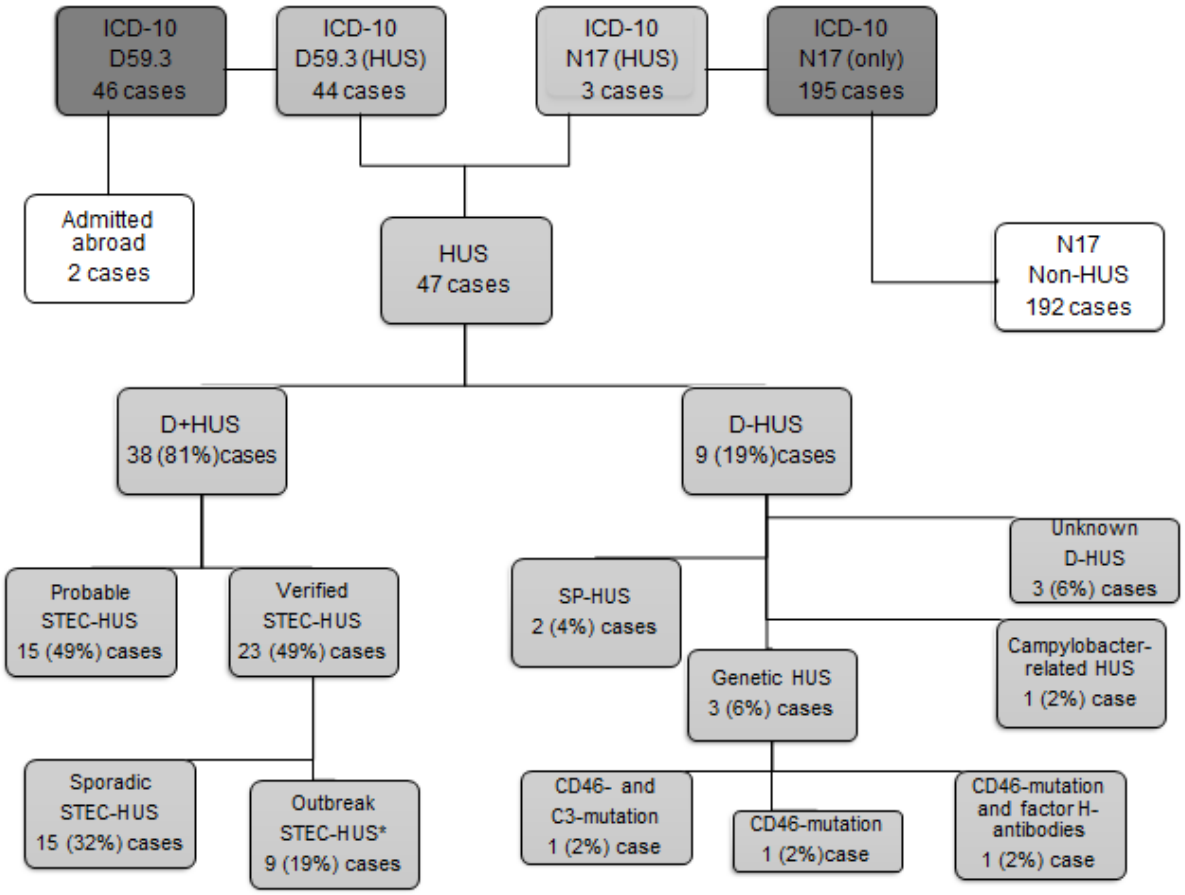
5. Summary of results

The summarized presented in the following papers were obtained from data collected according to the criteria and methods described above. They were published in four papers (I, II, III and IV) covering the different aims of this thesis.

5.1 Paper I

A total of 47 cases of hemolytic-uremic syndrome (HUS) in children < 16 years of age in Norway were identified from 1999 up to and including 2008; 44 through an ICD-10 code for HUS (D59.3) and 3 cases through screening of 195 identified cases diagnosed with acute kidney injury (AKI). Two additional cases with D59.3 registered were excluded as they were initially admitted for HUS abroad (Figure 2).

Figure 2 (Paper I, modified): Flow chart depicting identified potential cases[a] (dark grey, N = 241), identified cases not matching criteria for (white) and confirmed cases of HUS (light grey) distributed[b] according to etiology, in children, Norway, 1999-2008

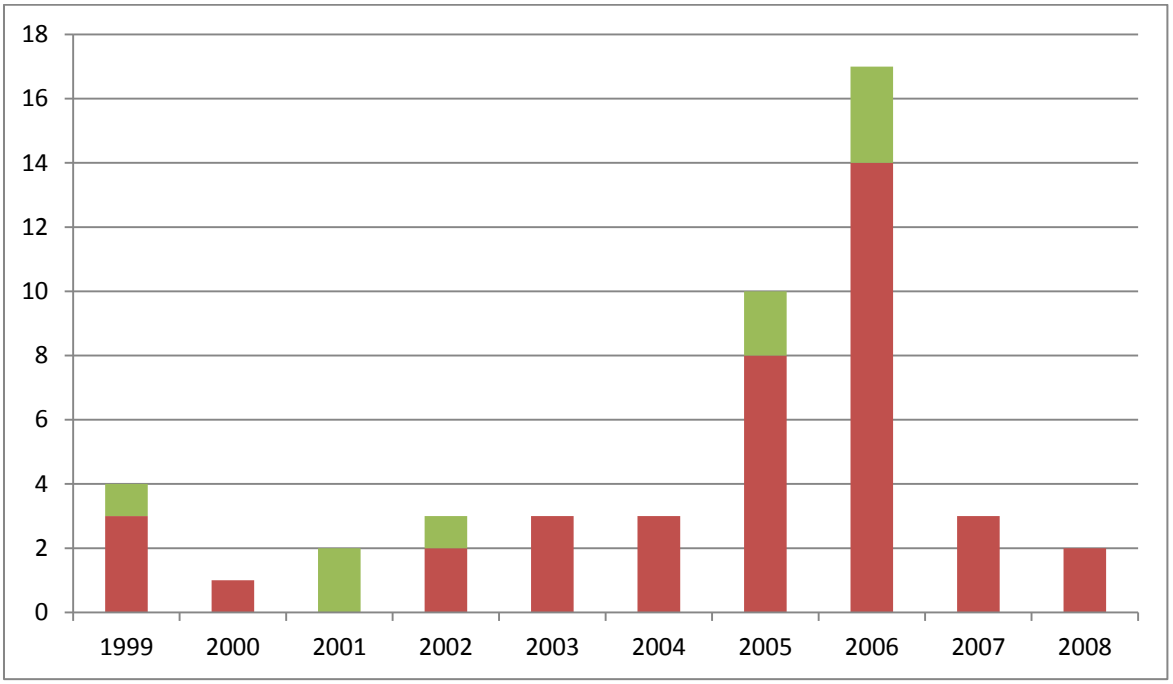


[a]AKI (N17) and HUS (D59.3). No HUS cases identified in nephritic syndrome group (N00/01/05)

[b]Presented as number of cases in each group and their respective proportion of the total number of identified HUS cases (%).

Thirty-one (66 %) cases were female. The yearly occurrence varied from one case (2000) to 17 cases (2006) (Figure 3). There were 38 (81 %) D^+HUS cases and nine (19 %) D^-HUS cases identified. The presence of Shiga toxin-producing *Escherichia Coli* (STEC) was verified in 23 cases (61 % of D^+HUS cases, 49 % of all HUS cases) (Figure 2).

Figure 3 (Paper I): Yearly occurrence of HUS, categorized into D^+HUS (red) and D-HUS (green) in children in Norway, 1999-2008



The average annual incidence rate for HUS was estimated to 0.5 cases per 100,000 children (range, 0.1-1.8) (Table 5). HUS occurred most often in the age group < 5 years, with 37 (79 %) of cases and an estimated average annual incidence rate of 1.3 cases per 100,000 children (range; lowest and highest year, respectively; 0.0-3.8). The average annual incidence rate for D^+HUS was estimated to 0.4 cases per 100,000 children (range; lowest and highest year, respectively; 0.0-1.4).

Table 5 (Paper I, modified): Annual age-spesific distribution and incidence rate (IR[a]) of HUS in children in Norway, 1999 and 2008

Type of HUS	D^+HUS; STEC-HUS	D+HUS; probable STEC-HUS	Total D^+HUS			D^-HUS		All HUS		
Measure / Age	Cases (N)	Cases (N)	N	%	IR[a]	N	%	N	%	IR[a]
0-4 y	19	11	30	79	1.0	8	89	38	81	1.3
0 y	2	1	3	8	0.5	2	22	5	11	0.9
1 y	7	5	12	32	2.1	4	44	16	34	2.7
2 y	5	3	8	21	1.4	0	0	8	17	1.4
3 y	0	2	2	5	0.3	0	0	2	4	0.3
4 y	5	0	5	13	0.8	2	22	7	15	1.2
5-9 y	3	3	6	16	0.2	1	11	7	15	0.2
10-15 y	1	1	2	5	<0.1	0	0	2	4	<0.1
Total	23	15	38	100	0.4	9	100	47	100	0.5

[a]Incidence rate (IR; average annual incidence rate in cases per 100,000 children)

Fourteen of the 23 verified STEC-HUS cases were sporadic cases. Excluding the nine outbreak cases with O103, O157 was the most commonly identified serogroup (five; 36 %). Other sporadic serogroups identified were two O103, two O26, two O145, one O87 and two were only non-O157/O103 was specified. Shiga toxins (Stx) were found in twelve (52 %) of the STEC-HUS cases; *Stx2* in ten cases and both *Stx1* and *Stx2* in two cases. The remaining eleven cases were considered STEC that had lost their toxin coding genes. Four were isolated from the 2006 outbreak cases. MLVA genotyping had been used to identify the causative agent in the absence of *stx*.

The remaining 15 D^+HUS (probable STEC-HUS) cases were classical HUS cases that had presented with diarrhea without verification of a causative agent. Follow-up through a minimum of one and a half years from initial hospital admittance was available and reviewed. None experienced recurrence of HUS during this period.

Nine D^-HUS cases were identified. Five were male. The average annual incidence rate for D^-HUS was estimated to < 0.1 cases per 100,000 children (range; lowest and highest year, respectively; 0.0-0.3). Eight (89%) of the nine cases were < 5 years of age. The remaining case was nine years.

Two D^-HUS cases were related to infection with *Streptococcus pneumoniae* (SP-HUS). Three cases had verified genetic mutations; all had a CD46 mutation, one had an additional C3-mutation and other antibodies to factor H. In one case without prodromal diarrhea, *Campylobacter jejuni* had been isolated and considered the

causative agent in the medical record. The remaining three cases were clinical HUS cases without prodromal diarrhea where no causative factor was identified.

In the study period, 28 HUS cases in children < 16 years of age were registered as notified to MSIS. Three MSIS cases were excluded and/or not included as individual cases as they had initially been admitted to hospitals abroad; one in 2003, the other in 2007. The former case had also been registered/notified twice (at different hospitals). In the same period, 102 cases of verified STEC infection (regardless of HUS development) were notified to MSIS in the same age group.

Twenty of the HUS cases registered in MSIS were notified from 1999 up to and including 2006. The remaining five were notified in 2007 and 2008. Seventeen of the cases notified before 2007 were identified in the STEC-HUS study group. The remaining three were identified in the 15 D^+HUS cases without verified etiology (probable STEC-HUS). These three were notified just before and after the outbreak in 2006. The five notified cases after 2006 were all identified in the STEC-HUS group. The remaining case in the STEC-HUS group had not been notified to MSIS. In the medical record search, 33 D^+HUS were identified before and five after 2007. The comparison between the MSIS and medical record cases is summarized in Table 6.

Table 6 (Paper I, modified): Difference in pediatric (<16 years of age) diarrhea-associated HUS cases notified to the Norwegian Surveillance System for Communicable Disease (MSIS) and identified in medical records in Norway, 1999-2008

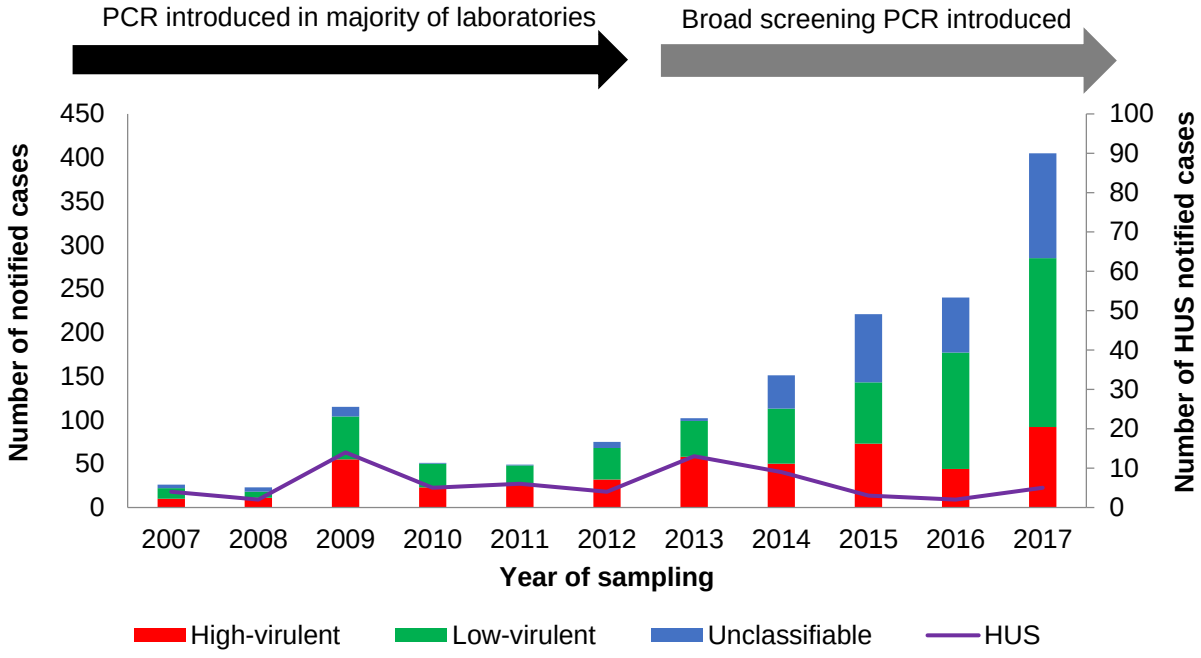
Identified by / Year	1999-2006	2007-2008	Total
MSIS	20	5	25
Medical records	33	5	38
Proportion reported to surveillance	61%	100%	66%

Based on the 23 STEC-HUS cases identified from medical records and assuming that all cases of STEC infection (regardless of HUS development) were notified according to guidelines, 23 % of the STEC cases notified to MSIS in the period were cases with HUS.

5.2 Paper II

There were a total of 1458 cases notified as STEC to MSIS (Figure 4). The median age was 21 years (range 0-97 years), 51% of cases were female, and most cases were ≤5 (37%). HUS was reported in 67 (5%) of cases, 5 of which were >15 years of age (range 25-81 years). Furthermore, where information was available, 25% reported bloody diarrhoea as the worst clinical outcome, 11% were asymptomatic, 26% were reported as hospitalized and 71% reported a domestically acquired infection. One or multiple stx subtype(s) was identified in 64% (936). The NRL received sample material for 1135 (78%) of the notified cases, but this proportion decreased over the study period, from 96% (324/339) in the years 2007-2012 to 72% (811/1119) in 2013-2017. The lowest yearly proportion was recorded in 2017 (64%, 260/405). The notified cases were categorised as; 475 (33%) high-virulent, 652 (45%) low-virulent, and 331 (23%) as unclassifiable STEC infections (Figure 4).

Figure 4 (Paper II, modified): Annual distribution of cases categorised with high-virulent, low-virulent or unclassifiable STEC notified to MSIS, 2007-2017 (N = 1458), and the number of HUS[a] cases (purple line, N = 67). The time periods when the majority of clinical medical laboratories in Norway introduced PCR detection of stx and implemented broad screening PCR in five of the laboratories are indicated with a black and grey arrow, respectively.



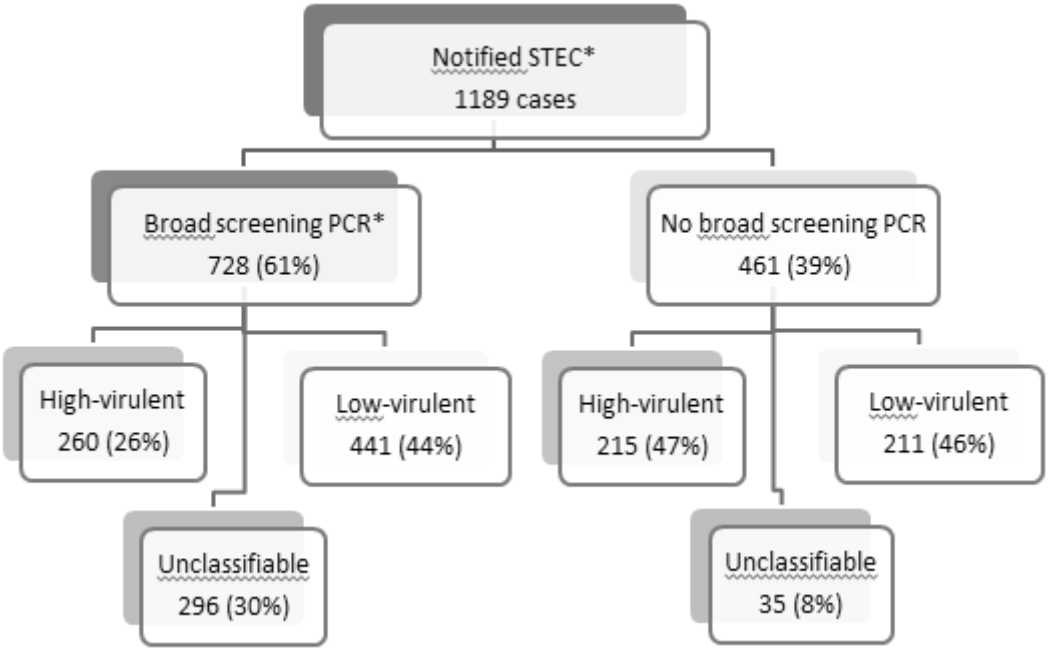
[a]Two national STEC-HUS outbreaks were reported during the study period, one in 2009 and one in 2013.

In children (<16 years of age), the estimated annual notification rate increased from 1.3 cases per 100 000 population in 2007 to 14.0 in 2017. In children ≤5 years of age, the estimated annual notification rate increased from 2.9 cases per 100 000 population in 2007 to 28.9 in 2017.

When comparing the high- and low-virulent cases, there were differences in the age distribution, with an estimated median age of 5 years (range 0-97 years) compared to 22 years (range 0-93 years) respectively ($p<0.001$), a higher proportion of cases with high-virulent STEC infections during summer (36% vs 29%) and less during winter (14% vs 21%) ($p=0.008$), and high-virulent STEC infection were more frequently reported as hospitalized than cases with a low-virulent infection (42% vs 21%, $p <0.001$). In the former group, the most commonly identified toxin gene subtypes were *stx2a* (224/403; 56%) and *Stx2c* (157/403; 39%), whereas *stx1a* (278/532; 52%) and *stx2b* (159/532; 30%) were more frequently seen in the low-virulent group. Additionally, virulence genes eae and ehxA were more prevalent in the high-virulent group (87% versus 51%, $p<0.001$ and 77% versus 51%, $p<0.001$, respectively). Furthermore, serogroups O157 (43% vs 1%), O145 (15% vs 5%), and O26 (17% vs 9%) were more commonly identified high-virulent STEC cases, while the opposite was observed for serogroup O103 (4% vs 23%) ($p<0.001$).

Through the 2017 survey and direct contact, we found that five medical microbiological laboratories implemented broad screening PCR during the study period, on the following dates: November 1st 2013, June 1st 2014, March 16th 2015, August 4th 2015 and April 1st 2017. The second laboratory had no record of notified STEC cases prior to 2013 and was therefore excluded from the times series analysis (*). The remaining 17 medical microbiological laboratories in Norway did not implement broad screening PCR during the study period. Distribution of cases is shown in Figure 5. The broad screening laboratory that was omitted from the analyses notified 30 high-virulent, 122 low-virulent and 117 unclassified STEC cases in the years 2013-2017.

Figure 5: Flow chart depicting categorized distribution of notified STEC cases notifed from laboratories that did and did not implement broad screening PCR (N = 1189). One laboratory was omitted from the overview as it had no cases notified prior to 2013 (*).



Adjusted for 1-year periodicity (significant in both models; sine-wave p<0.001, cosine-wave p<0.001), a higher increasing monthly trend in STEC cases (aIRR=1.020; 95% CI 1.016-1.024) notified from the four laboratories that had implemented broad screening PCR was observed, compared to laboratories that had not implement this method (aIRR=1.011; 95% CI 1.007-1.014, non-overlapping confidence intervals) (Figure 6). The difference in annual number of cases categorised as high-virulent, low-virulent or unclassifiable STEC infections was assessed in laboratories with and without broad screening PCR (Figure 7).

Figure 6 (Paper II): Monthly distribution of notified STEC cases with fitted trend based on times series analysis modela for the four medical microbiological laboratories that implemented broad screening PCR (N = 728 cases) and for the seventeen laboratories that did not implement broad screening PCR (N = 461 cases), Norway, 2007-2017. Time series analysis was conducted using negative binomial regression allowing for trends and for 1 year periodicity/seasonality. The different time points that the four laboratories started implementing broad screening PCR are marked with an asterisk (*).

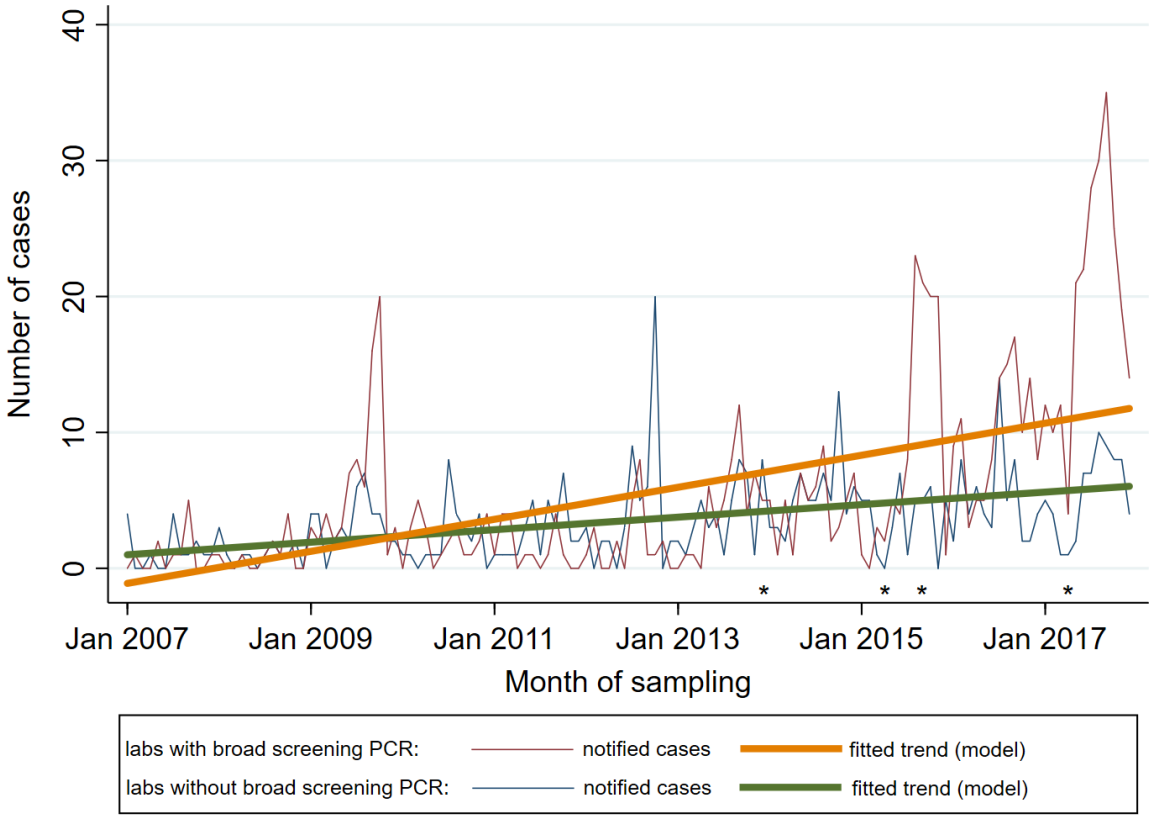
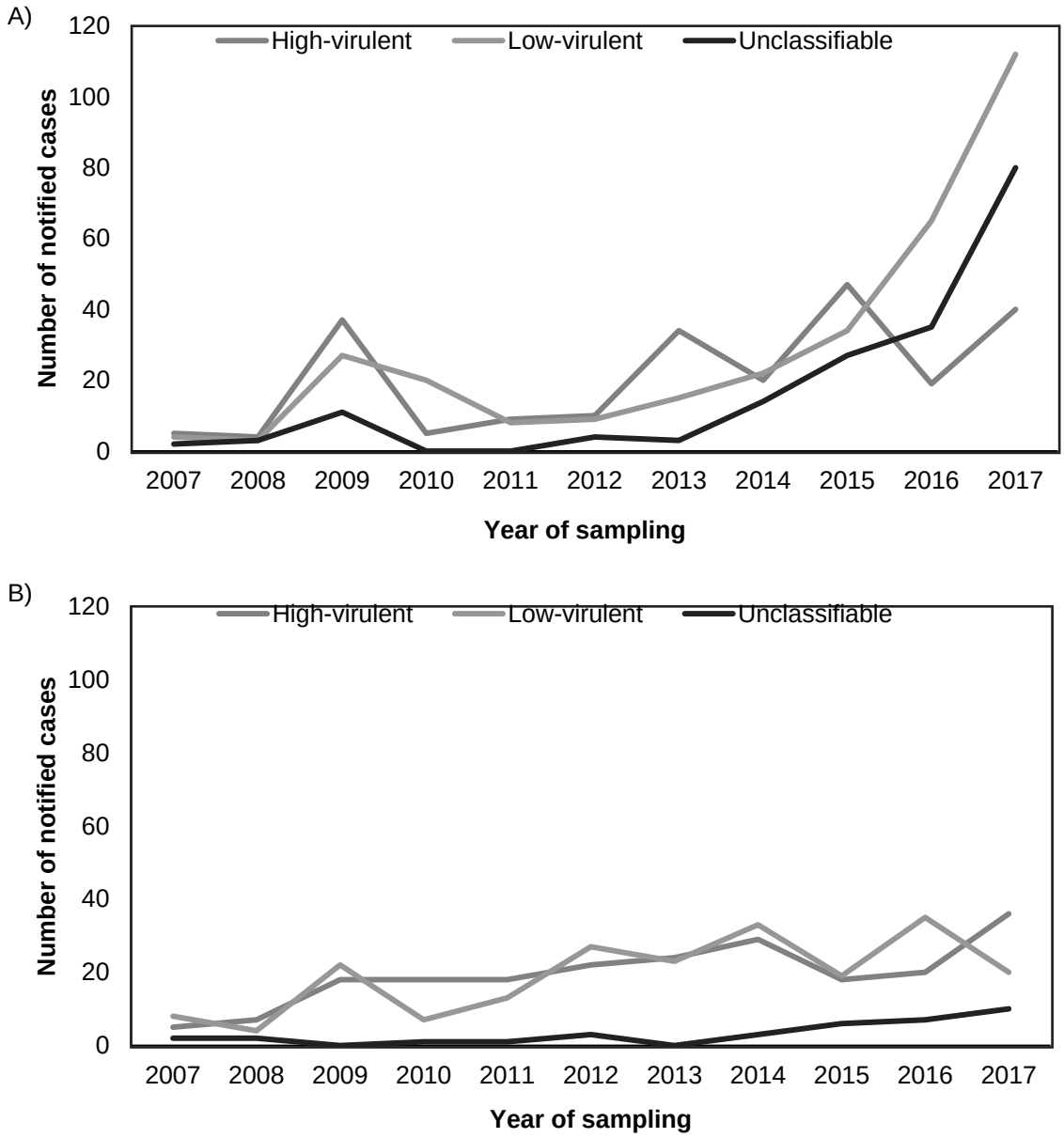


Figure 7 (Paper II): Annual distribution of cases notified and categorised with high-virulent, low-virulent or unclassifiable STEC infections from A) four laboratories that implemented broad screening PCR (N = 728) and B) seventeen laboratories that did not (N = 461), Norway, 2007-2017.



In the 997 STEC cases notified in the study period from all five laboratories that had implemented broad screening PCR, one or more concomitant bacteria was identified in 12% (112) of cases, increasing from 7% before to 15% after respective dates of implementation, while 44% of all concomitant bacteria were identified in the final year (2017). After the implementation of broad screening PCR, concomitant bacteria were identified in 11 (9%) cases with high-virulent, 26 (8%) cases with low-virulent and 59 (23%) cases with unclassifiable STEC infections. The most commonly identified group of concomitant bacteria was Campylobacter spp. (37%, 43/115 cases).

The follow up of surveillance of STEC and HUS through both Papers I and II provided an opportunity to expand on certain data. While the case definitions of Paper I restricted this to cases <16 years of age, this remains the group most affected by both conditions. Below I have provided data covering both study periods that was not presented similarly in either paper, but that are of interest in the context of this thesis.

Figure 8: Annual distribution of notified STEC cases that were verified as either O157 or non-O157 in children <16 years of age, in Norway, 1999-2017.

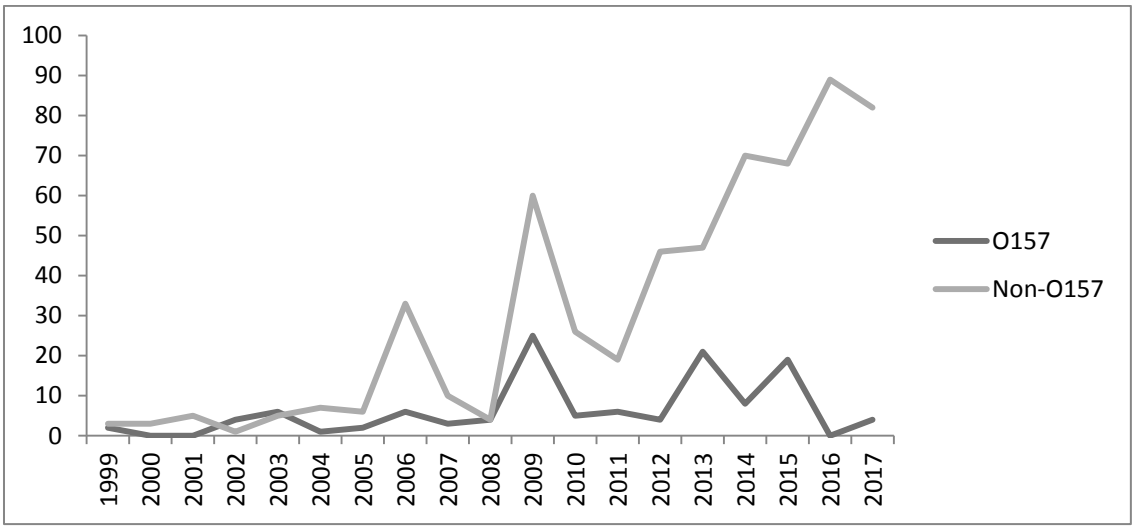


Figure 9: Annual distribution of notified STEC-HUS cases that were verified as either O157 or non-O157 in children <16 years of age, in Norway, 1999-2017.

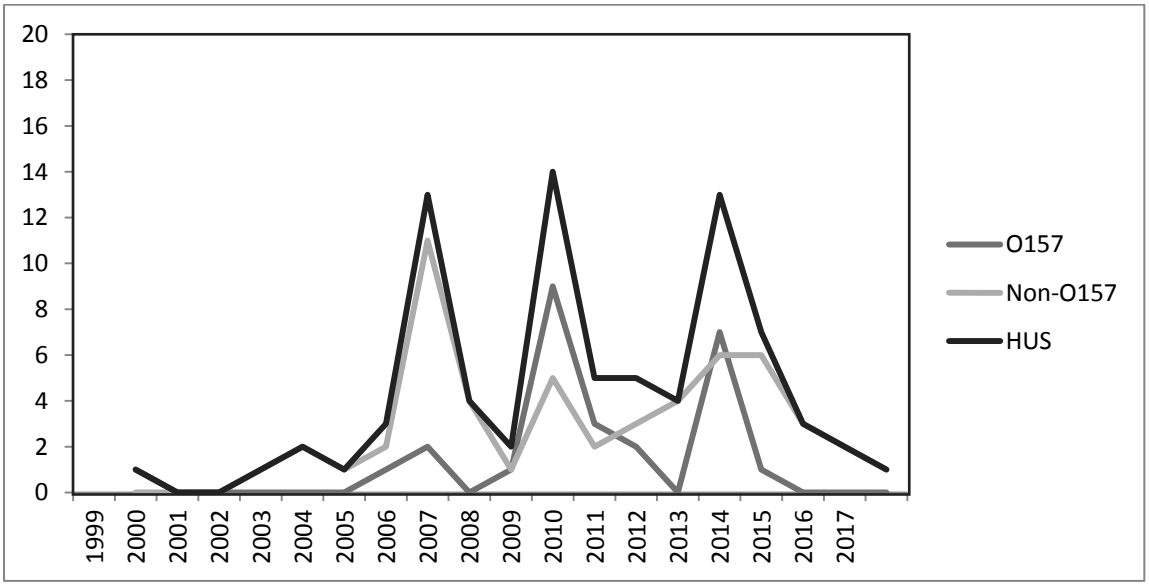


Table 7 (Paper I, modified): Geographical location, where available, of cases of STEC-HUS and non-HUS STEC according to associated health region, in children <16 years of age, in Norway, 1999-2017.

	STEC-HUS	Non-HUS STEC	Total
North	0	11	11
Middle	21	95	116
West	10	42	52
South-East	15	111	126
Unknown	0	10	10
Total	**46**	**269**	**315**

5.3 Paper III

The results obtained in Paper II depicted the clinical features and complications of HUS, related to both the acute phase and long-term oucome (Tables 8, 9, 10, 11 and 12).

Table 8 (Paper III): Clinical features and complications of HUS in children in Norway, 1999-2008. Results are presented as number and proportion of cases, N (%) and median (interquartile range). If data was not available in all medical records, the number of cases where available is presented with (N=number of cases where available).

Clinical feature	Diarrhoea-associated HUS (N=38)	Non-diarrhea-associated HUS (N=9)
Time first symptom to admittance (median, days)	6 (4-9)	5 (2-10)
Age at admittance (median, months/years)[a]	31 (range; 5 months-15 years)[a]	18 (range; 7 months-6 years)[a]
Duration of initial hospitalization (median, days)	15 (11-24)	16 (8-42)
Duration of total time hospitalized[b] (median, days)	18 (12-24)	16 (8-53)
Prodromal diarrhea (n, %)	37 (97%)	2 (22%)
Prodromal bloody diarrhea (n, %)	27 (71%)	2 (22%)
Hypertension at admittance (n, %)	4 (24%) (N=17)	2 (33%) (N=6)
Hypertension registered during admittance (n, %)	30 (83%) (N=36)	8 (100%) (N=8)
Oligoanuria (n, %)	29 (76%)	5 (56%)
Death acute phase (n, %)	2 (5%)	0 (0%)
Non-renal complications		
Neurological complications (n, %)	9 (24%)	2 (22%)
Cardiac complications (n, %)	2 (5%)	0 (0%)
Respiratory complications (n, %)	10 (26%)	2 (22%)
Gastrointestinal complications (n, %)	5 (13%)	1 (11%)
Pancreatic complications (n, %)	1 (3%)	0 (0%)
Sepsis (n, %)	11 (29%)	3 (33%)
Renal outcome/complications		
Proteinuria at first follow-up (n, %)	16 (50%) (N=32)	7 (78%)
Proteinuria ≥ 1 year after initial admission (n, %)	8 (38%) (N=21)	4 (57%) (N=7)
Hypertension at first follow-up (n, %)	10 (31%) (N=32)	5 (56%)
Hypertension ≥ 1 year after initial admission (n, %)	5 (26%) (N=19)	4 (80%) (N=5)
Chronic kidney disease (n, %)	2 (5%)	1 (11%)
End-stage renal disease (ESRD)	1 (3%)	0 (0%)

[a] Range; smallest and highest value for illustrational purposes.

[b] Time hospitalized including all readmissions for complications and extensive (not regular) follow-up

Table 9 (Paper III): Specified extra-renal complications in cases of D^+HUS in children in Norway, 1999-2008. N = total number of cases. Complications are presented with number of cases in which the complication was registered (n), from highest to lowest number of cases. Reported causative agent is specified in the sepsis group.

Complication group	N:	Complications (n):
Neurological complications	9	Seizures (4), mild brain infarctions (2), brain edema (2), brain microinfarctions (1), brain tamponade (1), meningitis (1), intracranial hematoma (1), anoxic brain damage (1), epilepsy (1), lowered white matter echogenicity (1), inability to remember words (1)
Cardiac complications	2	Multiple myocardial infarctions (1), cardiac arrest with resuscitation (1), pericardial fluid efflusion (1)
Respiratory complications	10	Acute respiratory failure (9), hydrothorax (3), pneumothorax (1), pulmonary collapse (1), chronic respiratory failure (1)
Gastrointestinal complications	5	Perforating colonic necrosis with peritonitis and hemi/subtotal colectomy (2), gall stone problems (2), intestinal invagination (2), rectal prolapse (1)
Pancreatic complications	1	Diabetes mellitus (1)
Sepsis	11	Unknown agent (4), *Staphylococcus aureus* (2), *Staphylococcus epidermidis* (2), *Acinetobacter baumannii* (2), streptococci (1), urosepsis of unknown cause (1)

Table 10 (Paper III): Specified extra-renal complications in cases of D^-HUS in children in Norway, 1999-2008. N = total number of cases. Complications are presented with number of cases in which the complication was registered (n), from highest to lowest number of cases. Reported causative agent is specified in the sepsis group.

Complication group	N:	Complications (n):
Neurological complications	2	Seizures (1), septic meningitis (1), brain atrophy (1), hemiplegia with spastic convulsions (1), epileptic activity (1), neuronal hearing loss (1), retinopathy (1)
Cardiac complications	0	
Respiratory complications	2	Acute respiratory failure (2), pleural empyema (1), septic pneumonia (1)
Gastrointestinal complications	1	Gall stone problems (1)
Pancreatic complications	0	
Sepsis	3	Pneumococci (2), *Staphylococcus aureus* (1)

Table 11 (Paper III): Therapeutic interventions in cases of HUS in children in Norway, 1999-2008. Results are presented as number of cases, n (%) and median (interquartile range). The values for type and duration of dialysis are estimated from those who received dialysis only (N). ERCP = endoscopic retrograde cholangiopancreatography.

Therapeutic interventions	Diarrhoea-associated HUS (N=38)	Non-diarrhea-associated HUS (N=9)
Dialysis – any type (n, %)	22 (58%)	3 (33%)
Type of dialysis (n)	(N=22)	(N=3)
– Peritoneal (n, %)	6 (27%)	1 (33%)
– Hemodialysis (n, %)	13 (59%)	2 (66%)
– Both (n, %)	3 (14%)	0 (0%)
Duration of dialysis (median, days)	8 (5-15) (N=22[a])	12 (7-13) (N=3)
Plasmapheresis (n, %)	3 (8%)	1 (11%)
Red blood cell transfusion(s) (n, %)	34 (89%)	9 (100%)
Platelet transfusion(s) (n, %)	15 (39%)	3 (33%)
Plasma infusion(s) (n, %)	6 (16%)	4 (44%)
Antibiotics – any indication (n, %)	23 (61%)	4 (44%)
Ventilation therapy (n, %)	9 (24%)	2 (22%)
ERCP (n, %)	0 (0%)	1 (11%)
Cholecystostomy (n, %)	1 (3%)	0 (0%)
Renal transplantation (n, %)	1 (3%)[b]	0 (0%)

[a] Including the only patient that received dialysis after initial admission (for an additional 133 days until renal transplantation)

[b] 12 months after initial admission

Table 12 (Paper III): Laboratory data in cases of HUS in children in Norway, 1999-2008. Results are presented as number of cases, n (%) and medians with interquartile ranges. If information on the feature was not available in all medical records, the number of cases where available is specified (N). Estimated glomerular filtration rate (eGFR) was estimated retrospectively using the height-independent Pottel eGFR equation (359). LD = Lactate dehydrogenase; CRP = C-reactive protein; WBC = white blood cell.

Laboratory feature	Diarrhoea-associated HUS (N=38)	Non-diarrhea-associated HUS (N=9)
Hemoglobin at admission (median, g/dL)	11.1 (7.8-12.7) (N=31)	6.7 (6.2-7.2) (N=7)
Hemoglobin, minimum value (median, g/dL)	6.5 (5.8-7,5)	6.0 (5.9-6.2) (N=8)
Creatinine at admission <1y (median, µmol/L)	35 (31-250) (N=3)	86 (61-110) (N=2)
Creatinine at admission ≥1y (median, µmol/L)	135 (61-275) (N=25)	115 (110-132) (N=5)
Creatinine, maximum value <1y (median, µmol/L)	231 (197-348) (N=3)	97 (67-126) (N=2)
Creatinine, maximum value ≥1y (median, µmol/L)	355 (200-465) (N=35)	228 (124-307) (N=6)
eGFR at admission <1y (median, ml/min/1,73m^2)	42.8 (23.0-49.1) (N=3)	21.8 (12.4-31.2) (N=2)
eGFR at admission ≥1y (median, ml/min/1,73m^2)	16.4 (11.0-58.5) (N=25)	19.4 (18.5-28.0) (N=5)
eGFR, minimum value <1y (median, ml/min/1,73m^2)	6.5 (4.9-7.8) (N=3)	21.6 (12.1-31.0) (N=2)
eGFR, minimum value ≥1y (median, ml/min/1,73m^2)	15.0 (6.3-13.8) (N=35)	13.9 (7.6-21.8)
LD[a] at admission (median, U/L)	2241 (1153-2728) (N=17)	2075 (1863-2659) (N=5)
LD, maximum value (median, U/L)	3146 (2559-4023)	3090 (2441-5931) (N=7)
Platelet count at admission (median, x10^9/L)	59 (39-175) (N=30)	39 (24-107) (N=7)
Platelet count, minimum value (median, x10^9/L)	32 (20-50)	24 (19-55) (N=8)
CRP[b] at admission (median, mg/L)	14 (9-30) (N=30)	13 (2-21) (N=6)
CRP, maximum value (median, mg/L)	67 (19-138) (N=37)	29 (15-161) (N=7)
WBC[c] count at admission (median, x10^9/L)	17.0 (11.2-25.4) (N=29)	11.6 (9.4-14.1) (N=7)
WBC count, maximum value (median, x10^9/L)	19.4 (15.1-29.4)	16.0 (14.4-17.4) (N=8)
Sodium at admission (median, µmol/L)	134 (130-137) (N=27)	135 (130-135) (N=6)

5.4 Paper IV

This paper aimed to estimate the occurrence, incidence and distribution of etiologies of AKI in children in Norway, and specifically assess the burden of HUS in AKI cases. In the study period, 315 cases of acute kidney injury (AKI) were identified in children < 16 years of age; 221 (70%) cases had an ICD-10 diagnose code for AKI (N17), 23 (7%) were identified through an ICD-10 diagnose code for HUS (D59.3) and the remaining 71 (23%) through an ICD-10 diagnose code for one of the nephritic syndromes (N00/N01/N05).

The median annual occurrence was 33 cases, and ranged from 17 cases in 2000 to 51 cases in 2006. The estimated average annual incidence rate for AKI was 3.3 cases per 100,000 children (range, 1.8-5.2). 148 (47 %) were female. The yearly occurrence AKI occurred most often in the age group <5 years, with 137 (43 %) cases and an estimated average annual incidence rate of 4.7 cases per 100,000 children.

Categorized according to probable pathophysiological mechanism, there were 75 (24%) prerenal, 234 intrinsic/renal (74%) and 5 (2%) post-renal cases (Table 13). The most common cause was the group consisting of nephritic syndromes (138; 44 %), followed by HUS (47; 15 %) and septicemia (8 %). The former group remained the most common cause when disregarding the 71 cases identified through their respective ICD-10 codes. From the 15% cases related to HUS, D^+HUS cases accounted for 12%, D^-HUS for 3 % and STEC-HUS for 7 % of all AKI cases.

Table 13: Distribution of etiology of AKI in children <16 years of age, in Norway, 1999-2008

Prerenal			Renal			Postrenal		
Aetiological group	N	%	Aetiological group	N	%	Aetiological group	N	%
Sepsis	24	7.6	Nephritic syndromes	138	43.8	Congenital anomalies of the kidney and urinary tract	3	1.0
Dehydration	23	7.3	Haemolytic-uraemic syndrome	47	14.9	Vesicoureteral reflux	1	0.3
Cardiological aetiologies	11	3.5	Oncological	16	5.1	Pelvic tumour	1	0.3
Medical/surgical complications	5	1.6	Drug related	8	2.5			
Systemic shock	2	0.6	Congenital anomalies of the kidney and urinary tract	7	2.2			
Drowning (multiple organ failure)	2	0.6	Genetic disorders	5	1.6			
Meningitis	2	0.6	Rhabdomyolysis	5	1.6			
Acute on chronic	1	0.3	Nephropathia epidemica	2	0.6			
Appendicitis	1	0.3	Unknown renal	2	0.6			
Encephalitis	1	0.3	Severe combined immunodeficiency	1	0.3			
Hypophyseal defect	1	0.3	Intoxication	1	0.3			
Diabetes complications	1	0.3	Wegeners granulomatosis	1	0.3			
Respiratory failure	1	0.3	Cerebral palsy complications	1	0.3			
Total prerenal	75	23.8	Total renal	234	74.2	Total postrenal	5	1.6
Unknown	1	0.3						

6. General discussion

In this thesis, different aspects of hemolytic-uremic syndrome (HUS) in children in Norway are described and discussed. The main data on HUS were obtained from medical records of children admitted to hospital during the period 1999 up to and including 2008. This was the decade where HUS emerged from being a relatively unknown condition in Norway, to reach the attention of the general public through media coverage of the first HUS death in 2004 and the first outbreak in 2006. HUS was a familiar and much studied condition in most of Europe and the US at this point. The emergence of HUS as a public health concern in Norway led to the undertaking of this study as the need for knowledge of the national HUS situation became apparent. In this section of the thesis, I will discuss and reflect upon the methodological considerations made throughout the study. Later, I assess the main findings of the study and conclusions.

6.2 Methodological considerations

6.2.1 Study design and time span

The catalyst for the initiation of the study comprising Papers I, III and IV was the national outbreak STEC and related HUS cases in 2006 (163), the first of its kind in Norway. While HUS had been under surveillance for several years, the outbreak highlighted the need for broader notification criteria. The study was performed to examine the national HUS situation. We also wanted to assess whether the new criteria had led to an increase in cases notified. This could suggest whether HUS was more frequent than previously assumed. We chose a retrospective study design to enable an assessment of HUS preceding and following the 2006 outbreak. It was also decided to examine the epidemiology of AKI, which had not previously been done on a national level. This would allow us to better assess the burden of HUS on a national level, as it is considered a major contributor to AKI in Europe (10), A nationwide study was realistic as both the base population and the expected HUS population would be relatively small. We assumed that the AKI population would be manageable within certain limits (discussed later), The number of relevant hospitals involved was limited.

We considered performing a prospective cohort study on both HUS and AKI. This would have allowed us to follow cases more closely and facilitate data access. It would also allow us to involve clinicians involved in the treatment of ongoing cases. These aspects were limited in our retrospective assessment of medical journal data. A prospective cohort study of HUS was theoretically manageable given the low number of cases expected per year. However, this coould also be a limitation; a prospective cohort study would in our opinion have to cover at least five years to account for variations in occurrence, especially due to outbreaks. The retrospective design reduced this effect as it allowed us to examine occurrence over ten years. The major hindrance

for initiation of a prospective study, especially on AKI, was restricted financial and administrative resources.

The study period for papers I-III could ideally have been expanded beyond 2008 to better accommodate assessment of the surveillance of HUS and STEC. The timing of study initiation made this difficult. After the public attention surrounding the 2006 outbreak, there was much interest in exploring the current status of HUS in Norway. Hovland and the candidate undertook and drafted the project in 2008 on a volunteer basis next to medical studies. Limited funds were in place and only allowed for remote data collection for a retrospective study as part-time funded project coworkers in 2009. It was thus decided to limit the study span to 2008, which would also allow for sufficient time for follow-up data from admission. The project was then successfully applied into the MSRP by the candidate as what would be considered a research project with an incomplete data set. Funding was not sufficient to undertake a new remote data collection tour to expand the study period, as this would have demanded extensive travelling. The amount of data it would generate and time required was also considered sufficient to publish three papers by the end of the MSRP period (one year full-time, two years part-time next to medical studies). Thus the project was kept as originally planned.

Upon rejection of the original thesis, funds had been made specifically available for a separate investigation on the changes seen in results from the STEC surveillance in Norway. This was considered suitable as an extended work to complement the original thesis, as it allowed for linking of surveillance data beyond the initial study, and thus constitute Paper IV. The time span of this paper, 2007-2017, was set to accommodate both a continuation of surveillance data in Paper I and the study objectives. A retrospective design was necessary to adhere to the study objective of comparing STEC surveillance across initiation of multiplex methods.

6.2.2 Classification and terminology

HUS has traditionally been classified by the presence (D^+HUS) or absence (D^-HUS) of prodromal diarrhea (33). This is generally thought to reflect the causality of HUS. Diarrhea usually precedes HUS caused by STEC infection and is often absent in the numerous "atypical" cases. However, it is well-documented that this is not always the case (33). A classification of HUS based on both verified cause and clinical association was proposed in 2006 and is increasingly used in clinical practice and research (6;8). This classification is an excellent tool for stimulation of a more thorough investigation. However, the former classification arguably still has important clinical implications. This is especially true in the initial acute phase, when critical therapeutic considerations may be necessary prior to causal verification. Its value is also reflected in the national notification criteria. Notification based on prodromal diarrhea may facilitate an early response to outbreaks and contribute to rapid inclusion of affected cases. The D^+/D^-classification was widely used at the initiation of this

study and still is today. These considerations led us to use the "classical" classification to better accommodate the aims of this study.

In the study protocol, we preferred the term enterohemorrhagic *Escherichia coli* (EHEC) to Shiga toxin-producing *Escherichia coli* (STEC). STEC are *E. coli* that produce Shiga toxins which potentially cause illness in humans, while EHEC are STEC that are usually pathogenic to humans (10;13;42). STEC is now the preferred term in international papers and guidelines, although EHEC is still infrequently used (35;42). EHEC is more commonly used in Norway, especially in infection surveillance (124). It was initially decided that EHEC would better describe the conditions in Norway. Following reviewers recommendations, it was decided to change this to STEC (and STEC-HUS) to accommodate international trends. This has no implication on the results or comparison to other countries as it is merely a terminological issue.

6.2.3 Case definitions – age limit

In the initial study, our pediatric population was defined as children below 16 years of age. General age limits exist to define the different stages of age, but these may differ according to culture, laws and general perceptions. This was an important factor to consider when comparing epidemiological studies on populations termed "children". The age limit of <16 years of age is frequently used in similar studies, although this varies from <15 to <17 (Table 1). HUS in children is most common in the age group <5 years (33). From this one must assume that the incidence rate (IR) generally decreases for each added base population year >4. Comparing the total IR of a study including children <15 years of age directly to a study with <16 would most likely be incorrect, or imprecise at best. It would be more fruitful to compare IR <5 years of age. This had to be considered in deciding our case definitions and when comparing to similar studies. Notably, the study population in Paper II was of all ages. While this necessitates a more general discussion of the results presented in the paper, this thesis provided an opportunity to present some of the data within the age limits of the initial study.

6.2.4 Case definitions – laboratory values

In Papers I, III and IV, one of the more challenging aspects was to determine a study definition of acutely reduced renal function in HUS and AKI cases. We considered using the p-RIFLE criteria that were introduced in 2007 (275). These are based on a decrease in estimated creatinine clearance and urinary output based on weight. Our pilot project revealed that data on both urinary output and weight were frequently missing or incompletely documented. This also applied to data on height, which excluded use of the estimated creatinine clearance Schwartz equation (285). We finally decided to use serum creatinine with limits based on current recommendations at that time after consulting our pediatric nephrologists. Measurements of serum creatinine is subject to some uncertainty (360) asit is influenced by a host of factors, including age,

weight, sex, diet, fluid administration, drugs and muscle mass (276;334;338). SCr levels are also usually not significantly elevated until 24-72 hours following renal damage (282). Reference values also differ between laboratories and methods applied. Of the relevant laboratories, only one kept the same method throughout the study period; the remaining laboratories changed method at least once, and similar metods also differed according to manufacturer. This was regrettably not taken into account account in the definition criteria. Strict upper limits were instead chosen to adjust for this and avoid overestimation of occurrence. This especially applied to the AKI cases. The strict limits resulted in a steep increase in defined serum creatinine when aged one year (or more) which would have excluded four clinical HUS cases from the study. In retrospect, this was a foreseeable problem that could have been avoided by expanding the age-related reference limits for serum creatinine. This issue is further addressed below.

The use of strict case definitions was also challenging when essential data were missing. In one patient, serum hemoglobin was only documented at admission, and at that time within normal reference limits. This is not uncommon in the early stage of HUS and may reflect serious dehydration or admission before the acute phase of hemolysis (361). This patient died in the acute phase and was clearly considered HUS. We included this case, although it did not technically fulfill the preset criteria for anemia (low serum hemoglobin).

It is unlikely that these issues have caused us to miss potential HUS cases, but could potentially mean that some AKI cases were not included. Our intention was to avoid overestimation and we consulted our expert pediatric nephrologists when in doubt. An alternative was defining cases by general clinical features not limited by laboratory values. This method has been applied in similar studies on HUS (25;31).

We consulted our expert pediatric nephrologists concerning the inclusion of such cases during the data collection process. These issues resurfaced and relevant cases were properly discussed in detail in Paper III, which presented data on clinical features and laboratory values. The previous papers had already been published at that time. In retrospect, this should also have been clearly stated in Paper I and IV.

6.3 Epidemiology and surveillance of HUS in children in Norway

One of the main aims was to investigate the epidemiological aspects of HUS in children in Norway. The average annual overall IR estimated (0.5 cases per 100,000 children) and occurrence was lower than previously expected due to the underreporting caused by previous notification criteria. However, the estimated IR in Norway is among the lower having been published when compared to studies from other European countries, the US, and Australia (Table 1). While these studies vary in terms of inclusion age, and a direct comparison have limited value, only Austria and

early reports from Italy show an IR lower than Norway in the population aged <5 years. A more recent study from Italy estimated an IR higher than that of Norway and increasing through the ten-year study period (30). It should be noted that this study concerns a northern region of Italy.

6.3.1 Epidemiology of D^+HUS

Although slightly lower (81%), the proportion of D^+HUS cases was in line with previous estimates in litterature (33). STEC infection was only verified in 61 % of all D^+HUS cases, which was low compared to that of available reports (17;27;30), with few exceptions (23). This may be explained by several factors. Firstly, one of the keys to verifying STEC infection is early stool sampling. HUS usually develops several days after the early phase of diarrhea and stool sampling is thus often performed after the patients have stopped shedding bacteria (2). Furthermore, verification of non-O157 STEC is generally more complicated than for infections of O157 origin. Before the 2006 outbreak, many Norwegian laboratories based STEC verification on culture-dependent diagnostics, primarily focusing on identification of O157 (55;124). This is reflected by the increase seen in Table 8, where verified caes of O157 have remained relatively stale, while there has been prominent increase in non-O157 from the years following the 2006 outbreak. This may have influenced the low verification rate in our findings. Another potential influencing factor is the reported low prevalence of STEC, especially O157, in ruminants such as sheep and cattle in Norway (151;152;362). Previous studies have shown an association between cattle density and prevalence of HUS (147;148). This point was further enforced by the fact that only five (36%) of verified STEC in sporadic cases in our study were O157, which is still considered the most common cause of HUS in the Western World (21;25;27;32). On the other hand, the emerging importance of non-O157 STEC has been extensively described (14;21;137). This is reflected by the predominance of non-O157 infections in identified in our study. In light of this, one could also reasonably assume that some of the included "probable STEC-HUS" cases identified in our study were caused by unverified non-O157 STEC.

6.3.2 Epidemiology of D^-HUS

The IR for D^-HUS (<0.1 cases per 100,000 children) was low, in line with similar estimates (363). While SP-HUS has been shown to cause up to 40 % of D^-HUS cases and is considered an increasing problem (260;261;264), only two cases were identified in the study period. This could indicate a low occurrence of SP-HUS in Norway, although reports suggest that the occurrence is often underestimated (263). Several possible explanations have been offered, such as a general lack of awareness of the condition, HUS misdiagnosed as or coexisting with the clinically similar diagnosis of disseminated intravascular coagulation, and the unavailability of a highly specific diagnostic test in a clinical setting (263;264). We did not screen patients with verified

SP infection for HUS, primarily due to the limited number of expected cases compared to those that would have to be screened. This may partly explain the low number of cases identified.

In one of out study cases, *Campylobacter* was specified as the causative agent of HUS. Few reports of similar cases exist in literature (364). Whereas it is well documented that various infections may trigger aHUS episodes (6;363), no verified genetic factors or HUS relapses were reported for this case. One could arguably have considered this a D^+HUS case, as it was caused by an infection presenting with diarrhea. In the more recent classifications of HUS, it would be classified along with STEC-HUS and SP-HUS as an infectious HUS case (6). Despite this, it was included as a D^-HUS case in accordance with our case definitions.

6.3.3 Surveillance of HUS and STEC

Our initial evaluation of the sensitivity of HUS and STEC surveillance between 1999 and 2008, found that only 61 % of D^+HUS cases were notified when dependent on STEC verification. All D^+HUS cases identified between December 2006 and the study end point had been notified, but there were only five, all verified STEC-HUS. While this showed an underestimation of the HUS occurrence prior to this study, no conclusions could be drawn on the effect of the change of criteria.

We furthermore compared the number of identified STEC-HUS cases to that of STEC cases notified to MSIS in the initial study period (1999-2008) to assess whether there had been an underreporting/-estimating of STEC. There are acknowledged limitations associated with routine STEC surveillance. Patients experiencing mild symptoms may not seek medical attention, and if so, submission of stool samples is unlikely (63). There are also previously discussed challenges with verification in stool samples taken in late stages of diarrhea, and laboratory practices and routines may vary (17;257). Due to these acknowledged limitations, HUS surveillance is in some countries used to monitor trends in STEC infections, based on the average probability of STEC to cause HUS (14;17). HUS develops in an estimated 8-15% of STEC cases (33;321). Based on this estimate and the presumption that all identified STEC-HUS cases had been notified as STEC enteritis in Norway, 23 % of notified STEC cases would have had developed HUS.

A HUS/STEC proportion of this magnitude could potentially have been explained by three factors; highly virulent STEC strains being more common in Norway, an overestimation of HUS cases, and/or an underreporting of STEC cases. Although there have been reports of HUS/STEC-proportions above 23 % in outbreaks caused by especially virulent strains (40), this was considered unlikely as most cases identified in this study were sporadic and it is unlikely that most or all are highly virulent. The second appears equally unlikely, as we identified 15 probable STEC-HUS cases with the classical clinical presentation; most of which were probably unverified STEC-HUS cases. This would have increased rather than decreased the

proportion. Thus, our results indicated that the high STEC-HUS/STEC proportion was most likely due to underreporting of STEC cases. This is supported by the results of a 2016 German study, where a computed estimate of the true STEC incidence based on notified HUS was 32.3-fold higher than incidence reported by STEC surveillance (249).

The predicted underestimation of STEC was seemingly justified by the results in Paper II, where we investigated an increase of STEC cases notified to MSIS in recent years. There was a significant overall increase throughout the study period, accentuated by a sharp increase after 2014, with similar observations reported in both Europe and the US (14;137). In our study, this was clearly more prominent in cases belonging to the low-virulent STEC group and largely attributed to cases notified from laboratories that had implemented broad screening PCR during the study period, as illustrated in Figure 7. Similar effects have been described after implementation of non-selective stool screening; in a Danish study, this resulted in an 88% increase of STEC in an associated laboratory (365). The annual number of notified HUS cases remained stable throughout the study period. Thus, based on the potential of HUS surveillance to monitor STEC occurrence, one would expect an increased detection rate of low-virulent STEC (i.e. not associated to HUS) in Norwegian laboratories when implementing unselected screening, as seen in our study. This likely reflects both the effect of a broader diagnostic approach and improved detection for non-O157 STEC over the last decade (137;365). It could also reflect an increased identification of asymptomatic carriers; a recent study showed an incidence rate of STEC infection in asymptomatic adults as high as 84.2 per 100 000 population, many of which belonging to O serogroups that were untypeable or rarely found in symptomatic patients (250).

The increase of low-virulent STEC poses a growing challenge to national STEC surveillance system, both in terms of labraotory capacity and socio-economic consequences for affected patients. In our study, we also observed a marked increase in notified cases where toxin subtype could not be verified, mostly *stx1/2* positive and culture negative, which is a common find with culture-independent STEC detection methods (366). The clinical impact of such cases is still unclear. While they may indeed represent STEC, they may simply suggest the presence of non-viale STEC (367), or identification of stx from free temperate bacteriophages (366). Studies also indicate that stx positive samples occur more frequently in cases with identified concomitant enteropathogens compared to cases with other common enteropathogens (367;368). This was reflected in our study, where concomitant baceteria were identified in 23 % of unclassified cases after implementation of unselected screening. These cases further challenge the STEC surveillance system, as no cultures are available to the national reference laboratories for further characterisation. In Norway, most of these cases would require to be followed-up as a probable high-virulent STEC infection until three consecutive stool samples are negative or a positive culture can

confirm a low-virulent STEC (258). This reinforce that while broad screening PCR techniques provide fast and sensitive identification and allow for rapid exclusion, it contributes to higher identification rates of both primary enteropathogens and concomitant bacteria and thus to an increased burden to public health services an those directly affected (13;367).

6.4 Clinical, therapeutic and long-term aspects of HUS

We also aimed to describe the clinical features of pediatric HUS patients included in the study. The results presented are mostly comparable to those seen in other studies, although certain findings deserve further attention.

One D^+HUS patient group presented without diarrhea, but was later verified as STEC-related and classified accordingly. Two patients in the D^-HUS group presented with diarrhea. One later had verified genetic HUS with relapses; the other was the Campylobacter-related case previously discussed. This underlines the weakness of a classification based purely on clinical presentation (6;33). I previously argued that this classification has advantages in the early stages of the acute phase and initial therapeutic considerations. This mainly applies to effects of early treatment with terminal complement inhibitors and plasma exchange therapy in suspected aHUS cases (200). These cases demonstrate that this approach may be misleading. On the other hand, a confirmed atypical presentation was only seen in one (4 %) of the verified STEC-HUS cases. This is low compared to the commonly cited proportion of up to 25 % (33).

6.4.1 The D^+HUS group

Short-term clinical features in the D^+HUS group were largely comparable to similar studies, both in terms of general features (time from initial symptom(s) to admittance, duration of hospitalization), renal complications (oligoanuria, hypertension), treatment modalities used (dialysis - duration, type and modality, supportive treatment), laboratory values and extra-renal complications (21;25-28).

The median value of serum hemoglobin at admission was 11.1 g/dL. Studies have shown that D^+HUS patients often present with hemoconcentration or normal hemoglobin values. It has also been suggested that hemoconcentration is a risk factor for CNS involvement and severe TMA activity (369). This had led to calls to change case definitions involving anemia to include signs of hemolysis as an alternative defining feature (369;370). The median value of LD at admission was 2241 U/L in our study. This points to early hemolytic activity and appears to support such a change. However, LD at admission was available in less than half of the cases and other markers of hemolysis were not registered. It is conceivable that LD was primarily analyzed in cases with a severe clinical picture. Therefore, nothing conclusive could be drawn from these data.

The use of strict values of sCR to define renal impairment in the lower age groups proved problematic. This was debated in the section concerning methodological consideration. Stringent use of our preset criteria would have excluded four evident HUS cases of low age. We decided to include these after consulting our expert pediatric nephrologists. This was problematized in Paper III, where a retrospective eGFR estimation based on the height-independent Pottel equation (359) was performed to further evaluate kidney function. These showed reduced age-specific kidney function in all patients involved. The Pottel equation has limited value when used retrospectively, and these estimations should be interpreted with caution.

The rate of long-term sequelae (persistent hypertension, proteinuria, chronic kidney disease) was comparable to similar studies (80;82). These results may have been overestimated as availability of information on long-term follow-up was limited in around half the cases. This could result from selective follow-up of more severe cases. Two patients died in the D^+HUS group, both in the acute phase. This corresponds with the widely reported case fatality rate of 3-5 % (33;321).

6.4.2 The D^-HUS group

We identified a low number of D^-HUS cases which were spread over several etiological groups. This made direct comparison of both the D^-HUS group and different subgroups to other studies difficult. Both SP-HUS cases were severely sick in the acute phase. One had serious long-term complications, none died. SP-HUS is usually associated with a severe course and high mortality in the acute phase, but a favorable long-term prognosis (5;260). Disease severity and mortality in the acute phase of genetic HUS (aHUS) vary according to type and penetrance of different defects. Long-term mortality and morbidity is usually high, but the prognosis is often unpredictable (270;274;371). aHUS is associated with a high need for dialysis and prolonged hospitalization (259). The renal complications documented in our study appeared less pronounced than described by Constantinescu et al (5). However, comparisons of subgroups are preferable as SP-HUS differ from the other etiologies described in this group.

6.5 Epidemiology and burden of HUS in AKI

The primary aims of Paper III were to present the epidemiology of AKI in children in Norway and estimate the proportion associated with HUS and its different subgroups.

6.5.1 Burden of HUS in AKI

HUS was the second most common cause of AKI in Norway. The most common cause was the group termed nephritic syndromes. Although limited by the study design and methodology, we were able to asses the burden of HUS in AKI based on a thorough nationwide medical record search. According to the European Centre for Disease Prevention and Control, HUS is considered the most common cause of AKI in

European children (321). However, few studies exist on etiology of AKI in different countries in Europe or worldwide. These are mostly based on patients from regions or centers/hospitals. All documented HUS as an important cause, but none of the reports identified it as the most common (301;302;372;373). A regional US study and a large single center study from Canada conversely reported HUS as the most common cause (324;374).

Certain aspects concerning these results should be noted. The primary cause of AKI was comprised in the parachute term “nephritic syndromes”, including different nephritis-related diagnoses. It was often difficult to separate these conditions by reviewing medical records, although most are clinically similar disease entities. The total number of cases was more than double that of HUS. A large proportion was identified through the screening of nephritis cases, but we also identified more nephritis cases than HUS cases diagnosed with AKI. This supports our conclusion.

6.5.2 Epidemiology of AKI

The main focus of the paper was the epidemiology of AKI. We estimated a national average annual incidence rate of 3.3 cases per 100,000 children. The number of AKI cases was likely markedly underestimated. For instance, only 51% of HUS cases had an additional ICD-10 code for AKI. This suggests that AKI diagnose codes may be left out when AKI is a part of a more prominent and/or severe clinical diagnose or a clinical syndrome. These cases were likely missed as we limited our search to the usual ICD-10 codes for AKI (N17), HUS and nephritic syndromes. Estimated pAKI occurrence may also be influenced by selected age limits; pAKI studies often exclude or differentiate the neonate subgroup or a subdivide patients below an age-limit of three months or less (316;318;320;323;329), due to the unique spectrum of neonatal AKI-related diseases (375). Patients below one year of age usually represent a substantial subgroup of AKI patients (280;318). Different lower age cut-offs thus likely contribute to some of the variation seen in epidemiological pAKI studies. This has likely contributed to the low rate seen in our study compared to that in others. An example is the high incidence of AKI seen in neonate asphyxia patients (278), a group we chose to exclude.

While we, to our knowledge, present the first publication to assess AKI epidemiology based on an assumed complete national cohort, there were clear limitations in our study. Similar studies have historically been based on experience from regions or centers and often depict specific patient groups, but have also indicated markedly higher occurrences (304;322;323). Nevertheless, it is widely considered that the incidence is increasing (278;376) and the need for international research on the various aspects of AKI has later been highlighted by the International Society of Nephrology's 0by25 initiative (297). However flawed, we hope that this paper may contribute towards a better understanding of a condition associated with high mortality and morbidity in children.

6.6 Strengths of the study

Regarding Papers I, III and IV, one of the major strengths was that we were able to perform a nationwide data collection. Our search for potential cases was performed at pediatric departments of all relevant hospitals in Norway. This meant that we could estimate national incidence rates rather than by extrapolation from national registries or regional centers. A relatively small base population and manageable distances were favorable factors. All data were collected by only two individuals working closely together and experts on pediatric nephrology were always available for consultation. This allowed us to continually and rapidly resolve potential discrepancies in what should be registered and how. We also minimized the risk of missing data through automatized extraction of datasets by direct and thorough assessment of each medical record in its entirety.

6.7 Limitations of the study

There were several limitations in this study. The limitations presented here partly overlap with reflections made in the methodological considerations section to expand on previous comments.

6.7.1 Study design

Through retrospectively collecting data from medical records for Papers I, III and IV, the results were subject to information bias. Most hospitals had changed from printed paper to electronic medical records during the ten-year study period. These differed from the electronic medical record systems applied in each hospital. The structure and content of both paper and electronic medical records are also subject to local standards, procedures and habits of the different hospitals. Clinical considerations documented by clinicians are also subject to their professional, objective and subjective opinions. Thus the availability, presentation and structure of data differed in several medical records assessed.

Measures were made to adjust for this bias. We predetermined precise definitions of the desired variables and used standardized abstraction forms to guide our data collection. The availability of variables and structure of the abstraction form was assessed through a pilot project in one of the relevant hospitals. This allowed us to adjust for missing data and interpretation of subjective data (e.g. from free text in medical record notes) to supplement parameters that were usually fixed (e.g. specified diagnose codes for complications supplemented by complications mentioned in free text). However, this generally did not allow for collection of data that was solely dependent on being mentioned in free text. This meant that we were unable to assess whether STEC-HUS patients had been infected abroad, as this would have depended solely on free text specifying exposure abroad. In contrast, patients admitted abroad were identified by a fixed section specifying where they were “admitted from”. Accordingly, while we could safely consider that all cases initially admitted abroad

were excluded, we could not identidy imported cases who had not been admitted there. Thus, the estimated incidence rates do not discriminate on location of exposure.

6.7.2 Case identification

Potential cases in papers I-III were identified by an initial medical record search of ICD-10 codes for HUS, AKI and nephritic syndromes. The use of administrative codes to identify cases has clear limitations. Reviews in Norway have repeatedly shown the limited quality of medical coding practices (377;378). AKI ICD-codes show high identification specificity (>95 %) in validation studies (305;379) and a high positive predictive value (95 %) was demonstrated when assessed with the KDIGO criteria (380). However, their limitations have repeatedly been shown through low identification sensitivity (305;379). An US study comparing the accuracy of AKI codes in large cohorts from 1994 and 2002, estimated an identification sensitivity of 17 and 29 %, respectively (305). A small pilot validation against RIFLE criteria found that while all 20 AKI coded patients fit the criteria, there were documented signs of AKI in 35 % (7/20) of non N17 coded sepsis patients and 4 % (2/50) in randomly selected (non AKI or sepsis coded) group (379).Despite the low sensitivity, the use of ICD-classification for case identification (and inclusion) is not uncommon in studies on AKI epidemiology (303;305;318). This is usually done to enable large cohorts. While our cohort would not be considered large in numbers by comparison, the intention was to establish a national cohort. Our medical record assessment approach eliminated some of the common limitations associated with diagnose code (registry) based epidemiology studies. It enabled us review each case by predefined inclusion criteria to exclude misdiagnosed cases. We were also able to identify readmissions (e.g. new episodes, transfer between hospitals) to avoid multiple case entries. It also allowed us to identify etiology based on clinical information rather than by additional diagnose codes.

The screening process could ideally have been widened to include other potential diagnose codes and patient groups in both HUS and AKI. This was underlined as we identified three HUS cases that were only registered as AKI. We considered hemolytic anemia, thrombocytopenia, STEC to further screen for HUS cases. The inclusion of patient groups such as dehydration/hypovolemia, sepsis and gastroenteritis would likely have been beneficial in the AKI paper. However, there were some obvious arguments to limit our search. One was the sheer workload and time required to thoroughly assess all medical records. Our design required complete examination of all available information in cases where complicated hospital stays often spanned several months. Data would also have to be gathered on-site, which required comprehensive travelling and associated costs. We thus had to adjust to the resources available and the anticipated gain in expanding our screening. In addition, several included hospitals implemented electronic medical record systems during our study period. Thus the medical records of a considerable proportion of relevant cases

had to be manually retrieved and returned from archives by hospital personnel. Inclusion of additional patient groups thus posed a notable workload for our local benefactors. There were also potential ethical issues to consider. Each added diagnose group would have required extended access to numerous patient medical records. The final extent of our screening was determined through these considerations and by consulting experts in the field of pediatric nephrology.

Information bias was also a limiting factor for case identification. We were dependent on the execution of routine measures in the various hospitals and associated laboratories. An example is absent detection of STEC in HUS patients. This could result from late sampling and microbiological diagnostic problems and limitations in Norwegian laboratories. This is suggested by the low rate of STEC-verified HUS compared to studies from other European countries. Fifteen cases were classified as probable STEC-HUS in our study; all diarrhea-associated cases with no identified cause or relapse(s). There were no further measures to adjust for this in a retrospective study.

The cases included in Paper II were predefined by their notification to MSIS, but the notification criteria for HUS may have impacted on our results in the context of this thesis. As mentioned, cases notified as HUS are registered as STEC under the common term "*E. coli*-enteritis – enterohemorrhagic disease, including D^+HUS". The notification criteria specify that this applies to HUS "in the context of acute diarrhea". However, in the case of an HUS case presenting without diarrhea but with verified STEC, it would still be notified a specified as HUS. Thus, we chose not to specify the HUS cases as diarrhea-associated in our definitions. Furthermore, the HUS criteria specify that a case should have "acute renal failure and at least microangiopathic haemolytic anaemia and/or thrombocytopenia". Accordingly, some of the HUS cases may have been what we referred to as "incomplete HUS" in the initial study. The comparison of HUS cases between Papers I and II should be viewed in light of this.

6.7.3 Data on follow-up

We were only able to retrieve documentation on follow-up in around half the cases in each group of the initial study. Several factors may have contributed to this. Procedures for follow-up of HUS may have differed between hospitals and/or have changed during the study period. Selective follow-up of more severely affected patients is another possibility. This may in turn have led to overestimation of long term sequelae. Many patients were also referred to follow-up at local hospitals. We contacted all relevant hospitals, but only a few had documentation of follow-up. It is possible that patients were further referred on to private clinics and/or their primary care physician.

In cases were documentation on follow-up was available from local hospitals, data were often limited or missing. This restricted our use of follow-up variables. It also made it necessary to identify CKD cases by diagnose codes rather than

acknowledged stage criteria (381). The proportion of CKD cases, especially those of lower stages, may subsequently have been underestimated. This is likely considering the number of patients with persistent hypertension and/or proteinuria.

6.7.4 Statistics

In Paper I and III, we considered performing comparative statistics between the two main groups, D^+HUS and D^-HUS. This was abandoned because of the low number and various etiologies in the D^-HUS cases. We also considered comparing certain features to outcome in the D^+HUS group, but again decided the group was too small for this to be fruitful. It should be noted that risk factors and predictors of severe disease have been thoroughly documented in previous studies (see Introduction). This resulted in mainly descriptive statistics being presented. These should always be interpreted with caution and prevented a more conclusive approach to some of our aims.

7. Conclusions

Hemolytic-uremic syndrome (HUS) has emerged as a recognized health concern in the last decades. This thesis presents a thorough examination of the epidemiology, clinical features and surveillance aspects of HUS in children in Norway. The knowledge provided is important for further understanding of a condition that primarily affects children, has severe, life-threatening complications for those affected.

The incidence of HUS in children in Norway was higher than previously assumed, but low compared to most other countries. This also applies to the frequency in which a STEC infection could be confirmed as cause. Possible contributing factors to these results have been proposed and discussed. The high proportions of short and long-term features reinforce previous depictions of a severe condition with a broad scope of systemic complications, which underline the importance of acute phase monitoring and thorough long-term routine follow-up.

Our initial assessment of the surveillance of HUS and STEC showed an underestimation of HUS occurrence when notification is dependent on microbiological confirmation of STEC. It also suggested an underreporting of STEC in general, which was reflected in the assessment of the increase in notified STEC cases in the years following the initial stufy. This was linked to a prominent increase in low-virulent STEC notified from laboratories that had implemented broad screening PCR methods. Rapid identification of STEC and STEC-HUS cases is essential for deciding the clinical approach and the prevention of outbreaks. While diagnostic procedures have improved in the aftermath of the 2006 outbreak, and with the later implementation of unselected screening techniques, they also contribute towards an increased burden to public health services and those directly affected. Our findings underline that clinicians should perform early stool sampling when STEC infection is suspected. They also highlight the need for reinforcement and continuous evaluation of the mandatory notification and surveillance of both HUS and STEC infections, while maintaining differentiated control measures for STEC cases to avoid costly follow-up of low-virulent STEC infections. Furthermore, our results suggest the need for further research towards a cost-effective broad screening PCR strategy that enables differentiation of high-virulent STEC infections.

This thesis has also described the epidemiology of acute kidney injury (AKI) in children on a national basis, although through highlighting the many limitations involved in epidemiologic reasearch. As the second most common cause of AKI in Norway, HUS contributes to the increasing occurrence of a condition that is considered an important contributor to mortality and morbidity in pediatric patients worldwide. This highlights the need for more knowledge on both HUS and AKI.

Our results and this thesis will hopefully contribute to the general understanding of HUS, both inside and outside the borders of Norway.

8. Future studies

This thesis presents a retrospective study over a period of ten years. The subject of HUS in Norway could ideally be further explored through a prospective study. This could be performed in children only or include adults. It would preferably be carried out over a period of several years due to the low yearly occurrence. The relatively low number of new cases per year also means it would be possible to perform on a nationwide basis. This could be used for comparison to the present data to uncover whether the national incidence is increasing or decreasing. A study of such magnitude would require a national network of clinicians and preferably have its center at one of the university hospitals. This would essentially function as a national HUS registry. Similar networks exist abroad, predominantly based in regional institutions. A potential cooperation across borders could contribute greatly in terms of experience with organizing and sustaining a registry.

There is increasing interest in research on AKI epidemiology internationally and our research recently garnered positive attention from other pediatric nephrologist It would be interesting to conduct a similar prospective study/registry on AKI in near future. Knowledge on this area is scarce, as discussed above. The few studies that exist on the etiology of AKI are often limited to regions, centers or hospitals. These are likely subject to discrepancy due to selection bias. National studies on conditions with a low yearly occurrence are conceivable in Norway due to the limited number of hospitals, as our research has shown. The design would be similar, but obviously require more in terms of resources and compliance from associated clinicians.

Through exploring the vast current knowledge on HUS, other areas of interest have appeared. Research on treatment of HUS has been broad, yet has until recent years mainly served to exclude option. No curative options existed until Eculizumab revolutionized treatment of D^-HUS patients. This has not been for a lack of effort to identify means to improve the prognosis of HUS patients. Antibiotics have been much debated and are currently advised against, despite claims that nuance this view. Several specific agents and vaccines have proven ineffective. Even Eculizumab, now on the way to being critical in D^-HUS patients, have shown varying effect in D^+HUS. , Targeted fluid therapy is the closest we have come in terms of prevention of HUS development. Although vastly explored in literature, more answers are bound to be out there. This would be an interesting area to explore in future work.

HUS is still a rapidly expanding field. It has gained pace in recent years following the advances on the role of the complement system in HUS pathophysiology. The gaps in AKI research also leave much to be desired. There are most certainly vast opportunities to explore in these fields, both for experienced and aspiring researchers.

Furthermore, the latest addition to this thesis (Paper II) has paralleled the implementation of WGS as a near future primary method for surveillance in Norway. The vast potential of WGS in surveillance, not only of STEC but also of other patoghens, is certain to create an array of opportunities for future research, especially considering that it in a historical context is still in its infancy. Meanwhile, as WGS will remain a vital tool for reference laboratories, there is still a huge potential in improving more traditional (and novel) detection methods. The rapid increase of detected STEC is fastly becoming a puclic health concern and methods to counter the increased workload are highly warranted.

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Ref Type: Online Source

10. PAPERS I-IV

PAPER I

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Incidence and etiology of hemolytic-uremic syndrome in children in Norway, 1999–2008 – a retrospective study of hospital records to assess the sensitivity of surveillance

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Abstract

Background: Public awareness of hemolytic-uremic syndrome (HUS), especially related to Shiga toxin-producing *Escherichia coli* (STEC), has increased in Europe in recent years; accentuated in Norway by a national outbreak in 2006 and in a European context especially by the 2011 outbreak originating in Germany. As STEC surveillance is difficult due to diagnostic challenges in detecting non-O157 infections, surveillance of HUS can be used to indicate the burden of STEC infection. Until 2006, notification of HUS to the Norwegian Communicable Disease Surveillance System (MSIS) was based on microbiologically confirmed infection with enterohemorrhagic *Escherichia coli* (EHEC), humanpathogenic STEC. In 2006, diarrhea-associated HUS (D^+HUS) was made notifiable based on clinical criteria alone. The incidence and etiology of HUS in children in Norway has not previously been described.

Methods: In order to assess the sensitivity of STEC and D^+HUS surveillance and describe the incidence and etiology of HUS in children in Norway, we conducted a nationwide retrospective study collecting data from medical records from pediatric departments for the period 1999–2008 and compared them with data from MSIS. Descriptive statistics are presented as proportions, median, average and mean values with ranges and as incidence rates, calculated using population numbers provided by official registries.

Results: Forty-seven HUS cases were identified, corresponding to an average annual incidence rate of 0.5 cases per 100,000 children. Diarrhea-associated HUS was identified in 38 (81%) cases, of which the median age was 29 months, 79% were <5 years of age and 68% were girls. From 1999 to 2006, thirteen more diarrhea-associated HUS cases were identified than had been notified to MSIS. From the change in notification criteria to 2008, those identified corresponded to those notified. STEC infection was verified in 23 (49%) of the HUS cases, in which O157 was the most frequently isolated sporadic serogroup.

Conclusions: Our results show that the incidence of HUS in children in Norway is low and suggest that D^+HUS cases may be underreported when notification requires microbiological confirmation. This may also indicate underreporting of STEC infections.

Keywords: Enterohaemorrhagic *E. coli* - EHEC, Epidemiology, Haemolytic uraemic syndrome, Surveillance, Shiga toxin producing *E. coli* - STEC

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Background

Hemolytic-uremic syndrome (HUS) is a clinical syndrome characterized by microangiopathic hemolytic anemia, impaired renal function and excessive platelet consumption leading to thrombocytopenia [1]. HUS is considered to be the most common cause of acute kidney injury (AKI) in European children, mainly affecting pre-school-aged children [2-4]. In the long term, HUS is associated with complications such as hypertension and end-stage renal disease with a death rate of approximately 3–5% [3]. Based on clinical presentation and probable etiology, HUS is commonly divided into diarrhea-associated HUS (D^+HUS) and non-diarrhea-associated HUS (D^-HUS), also called atypical HUS. Recent classifications have suggested a more specific approach based on causality and clinical associations [2]. Usually, around 90% of cases are D^+HUS, while 10% are atypical HUS [5-9].

The most common cause of D^+HUS in children is infection with Shiga toxin-producing *Escherichia coli* (STEC) [1], although not all STEC-associated HUS (STEC-HUS) cases present with diarrhea [7,10-12]. Shiga toxins are produced and released by STEC bacteria and are the main cause of STEC-HUS [1]. There are two main types of Shiga toxins, Shiga toxin 1 and Shiga toxin 2 (Stx 1 and 2). The latter is more frequently found in bacteria causing HUS than Shiga toxin 1. Ruminants are the main reservoir for STEC and infections are mainly food or waterborne. In D^-HUS, the most common causes are infection caused by *Streptococcus pneumoniae* (SP-HUS) and genetic forms of HUS [9].

HUS and STEC infections are under epidemiological surveillance in the EU. In 2012, the European Commission updated the European case definition for STEC, requiring laboratory confirmation of Stx or *stx* gene(s), except when STEC O157 is directly isolated [13]. Surveillance of HUS and STEC in many countries is based on this case definition, requiring laboratory confirmation prior to notification. There are certain challenges in the surveillance of STEC. Far from all STEC infections are treated by a physician, identification of the infectious agent in mild infections might be judged unnecessary by clinicians and until more recent years, verification of non-O157 serogroups was difficult. Because of this, some countries, including France, have previously used surveillance of HUS to follow trends and identify outbreaks of STEC infection [14]. This is based on STEC being the causal factor in most HUS cases. In 2009, 3573 cases of STEC were reported in the EU, of which half were caused by serogroup O157 [4]. In Europe and America, this serogroup is most frequently isolated from HUS patients [2,4,6,12,15], while O111 is dominant in Australia [16]. Serotype O157:H7 is the most easily diagnosed serotype, based on its failure to ferment sorbitol within 24 hours of incubation [17,18]. However, in recent years, changes in diagnostic procedures have led to the isolation of an increasing variety of non-O157 STEC serogroups from HUS patients in Europe, including O26, O91, O103, O111, O113, O121, O128, O145 and sorbitol-fermenting O157 (SF O157) [4,7,8,14,19]. Recent outbreaks of STEC infection in Europe, with varying degrees of HUS development, have resulted in increased awareness. In 2011, *E. coli* O104 caused a large outbreak with many HUS cases in Germany. While the majority of cases occurred among adults, it also affected more children than previously seen in any other European outbreak to date [20]. In Norway, a national outbreak of STEC O103:H25 occurred in 2006, in which nine children developed HUS, including one with fatal outcome [21,22], raising awareness among physicians as well as microbiological laboratories.

Notification of EHEC infections to the Norwegian Surveillance System for Communicable Diseases (MSIS) has been mandatory since 1989 [18]. In Norway, all clinicians and microbiological laboratories analyzing human specimens are required by law to notify cases of group A infectious diseases, such as STEC infection, to MSIS at the Norwegian Institute of Public Health. Prior to the Norwegian outbreak in 2006, notification was only required for HUS cases with laboratory-confirmed STEC infection. In December 2006, after the outbreak, notification criteria were changed to include all D^+HUS cases, based solely on clinical presentation. Since microbiological confirmation of STEC-HUS takes on average 14 days (measured from the day the stool sample is taken to the day the case is reported), the change in notification criteria aimed to improve the timeliness of reporting. In addition, clinically-based notification criteria were expected to increase the sensitivity of the surveillance system, as detection of STEC in a stool sample can be difficult and the bacteria may not be present at the time a patient develops HUS. Measures to improve diagnostic procedures were also implemented after the 2006 outbreak, and the laboratories methodology was gradually expanded to include PCR screening for presence of *stx* at all the microbiological laboratories. After implementation, ring tests sent out to all laboratories in the following years showed improved diagnostic capabilities for non-O157 serogroups.

The main aim of this study was to determine the annual incidence of D^+HUS among children <16 years of age diagnosed in Norway from 1999 up to and including 2008 through a review of medical records, compare these numbers with cases reported to MSIS, and consequently assess the sensitivity of the D^+HUS surveillance. The secondary aim was to describe annual incidence and etiology of all types of HUS in the same age group and for the same time period.

Methods

Design and data collection

We performed a retrospective, descriptive study. Data were collected from medical records from 24 pediatric

departments of Norwegian hospitals from patients <16 years of age admitted from the 1st of January, 1999, to the 31st of December, 2008. All hospitals with capacity and competence for supportive care of HUS and/or AKI patients were included.

Potential cases were identified by performing medical record searches for pediatric patients tagged with ICD-10 codes D59.3 (HUS), N17 (AKI) and/or N00/N01/N05 (acute nephritic syndrome/rapidly progressive nephritic syndrome/unspecified nephritic syndrome). Apart from D59.3, the diagnostic codes were investigated to identify potentially misdiagnosed cases of HUS. Only cases matching the case definitions were included, regardless of ICD-10 code. Medical records were assessed in both electronic and paper form. Data were registered in forms made in EpiData (www.epidata.dk), which were designed through a pilot project to determine the availability of desired variables in standard medical records. Data files were encry pted according to the information security standards of the Norwegian Institute of Public Health. Data on cases of HUS and STEC notified from the 1st of January, 1999, to the 31st of December, 2008, were exported from MSIS. Population figures for children <16 years were acquired from Statistics Norway (SSB).

Case definitions

A hemolytic-uremic syndrome (HUS) case was defined as:

- a case clinically compatible with all the following laboratory findings of
  - o thrombocytopenia (<150 × 10^9/L)
  - AND
  - o anemia (Hgb < 10.5 g/dL)
    - of hemolytic origin, with elevated serum LD (>500 U/L)
    - AND
  - o acutely reduced renal function (serum creatinine >35 μmol/L for patients < 1 years of age, > 80 μmol/L for patients 1-15 years of age)
  - AND
  - o Either
    - reported presence of fragmented red blood cells (schiztocytes) on peripheral blood smear; a sign of microangiopathic changes consistent with hemolysis, an important part of HUS pathophysiology [1]
  - OR
    - if peripheral blood smear was missing in the journal; probable clinical HUS confirmed by consulting a clinician with expertise in pediatric nephrology.

A diarrhea-associated HUS (D^+HUS) case was defined as a HUS case with either:

- a clinical presentation of prodromal diarrhea, without verifiable causative etiology (probable STEC-HUS).

or

- STEC-HUS, defined as a HUS case with laboratory-verified STEC-infection.

A D^-HUS case was defined as any non-diarrhea-associated HUS cases of non-STEC causality.

Microbiology

Information on microbiological findings was gathered from medical records and from MSIS for the notified cases. MSIS receives data on microbiological characteristics from the regional laboratories as well as from the National Reference Laboratory for Enteropathogenic Bacteria in Norway.

Statistical analysis

Calculations were performed using Microsoft Excel. Descriptive statistics are presented as proportions, median, average and mean values with ranges and as incidence rates, calculated using population numbers provided by official registries.

Ethical considerations

The study was approved by the Regional Ethical Committee South East A. Dispensation was granted from patient confidentiality regulations as potential participants would only be identifiable following the review of medical records. It was therefore not necessary to contact all potential cases to gain their consent prior to collecting journal data. However, once cases were identified through the medical journal review process, the parents were notified and could elect to withdraw from the study. No patients chose this option.

Results

Sensitivity of the D^+HUS and STEC surveillance

In the period 1999 up to and including 2008 28 HUS cases among children <16 years of age were notified to MSIS. Three cases, that is one case registered twice (two different hospitals) in 2003, and one in 2007 were identified and excluded from this study as they were initially admitted to a hospital abroad. In the same period, 102 cases of STEC infection were notified in the same age group. We identified 23 cases of STEC-HUS in medical records in the study period (Figure 1). Accordingly, 23% of the STEC-cases notified to MSIS in the period were cases with HUS.

Twenty of the HUS cases in MSIS were notified from the start of the study period up to and including 2006, and five after 2006. 17 of the cases notified before 2007 were identified as STEC-HUS cases; the remaining three were identified as probable STEC cases. These three were admitted to hospital just before and after the outbreak in 2006, thus probably notified as potential outbreak cases. The five cases

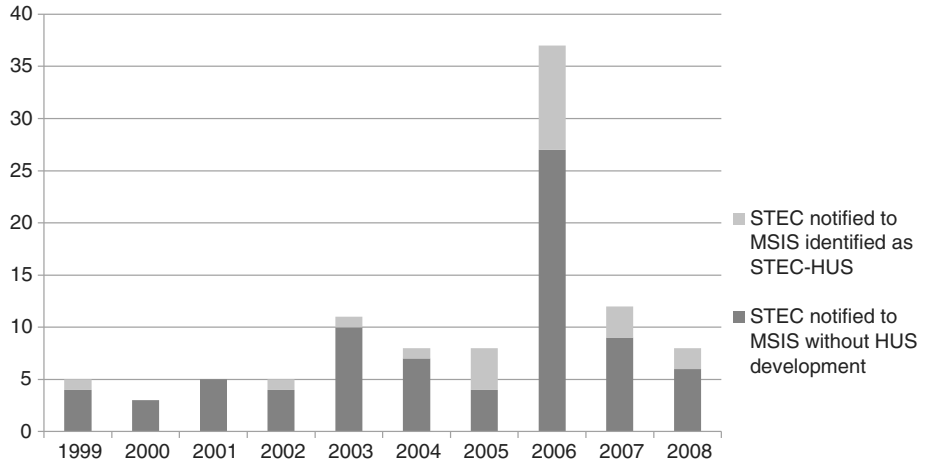

Figure 1 HUS associated with STEC cases in children in Norway notified to MSIS between 1999 and 2008. Cases of shiga toxin producing *E. coli* (STEC) infection reported to the Norwegian Communicable Disease Surveillance System, with share of cases identified in medical records as associated to hemolytic-uremic syndrome (HUS) in children <16 years of age, Norway, 1999–2008 (n = 102).

notified after 2006 were identified STEC-HUS cases. The remaining STEC-HUS case, from 2005, was not notified to MSIS. The corresponding numbers identified in the medical records search were 33 and five for the period 1999–2006 and 2007–2008, respectively (Table 1).

Incidence and etiology of all types of HUS in children 1999–2008

Based on information from the medical records, a total of 47 cases of HUS in children were identified from 24 different Norwegian hospitals in the period 1999 to 2008 (Figures 2 and 3), varying from one case (in 2000) to 17 cases (in 2006) per year (Figure 3). Of the 47 HUS cases, 44 had the diagnostic code D59.3 (HUS). Three cases, two probable STEC-HUS and one SP-HUS, were identified as HUS through the diagnostic code for acute kidney injury (AKI); N17. These were all recognized as HUS in the journal, but had been given the wrong diagnose code. All three fit the inclusion criteria.

The average annual incidence rate of HUS of any etiology was estimated to be 0.5 cases per 100,000 children (range; lowest and highest year, respectively; 0.1-1.8). Thirty-one (66%) were female. The incidence rate was highest in children <5 years of age, with an estimated average annual incidence rate of 1.3 cases per 100,000 children (range; lowest and highest year, respectively; 0.0-3.8) (Table 2). The highest proportion of cases was in children aged one year, accounting for 34% of the cases (Table 2). The median age at initial admission was 29 months (range, 5 months-15 years).

Table 1 Diarrhea-associated HUS cases in children in Norway notified and identified in children in Norway

Identified by/Year	1999-2006	2007-2008	Total
MSIS	20	5	25
Medical records	33	5	38
Proportion reported to surveillance	61%	100%	66%

Cases of diarrhea-associated hemolytic-uremic syndrome in children <16 years of age reported to the Norwegian Communicable Disease Surveillance System (MSIS) and identified through medical record search, Norway 1999–2008.

Based on clinical presentation, cases were categorized into 38 (81%) D^+HUS cases and 9 (19%) D^-HUS cases (Figure 2). In the medical records, results from stool examination were available for 43 (91%) patients and for serological testing for 28 (60%) patients. STEC infection was detected in 22 (51%) of the stool samples and seven (25%) of the serological samples. One or both of these tests were performed in 44 of the cases, and STEC infection was confirmed in 23 (52%) of them, thus in one case STEC was only detected by serological testing.

Of the 38 D^+HUS cases, 29 (76%) were sporadic and nine (24%) were outbreak cases; all nine cases were from the 2006 outbreak. The estimated average annual incidence rate for D^+HUS was 0.4 per 100,000 children (range; lowest and highest year, respectively; 0.0-1.4) (Table 2). Twenty-six (68%) were female. The estimated average annual incidence rate for D^+HUS was highest among children <5 years of age with 1.0 per 100,000 children (range; lowest and highest year, respectively; 0.0-3.5). This group constituted 30 (79%) of the D^+HUS cases. STEC was confirmed in 23 (61%) of the 38 D^+HUS cases. The remaining 15 cases presented with diarrhea, but without verified STEC infection or etiology and thus classified as probable STEC-HUS. In these cases, follow-up was evaluated in available medical records for a minimum of 1.5 years from first hospital admittance; none experienced recurrence of their HUS during this time.

The distribution of serotypes of STEC isolated from the 23 laboratory confirmed cases, is shown in Table 3.

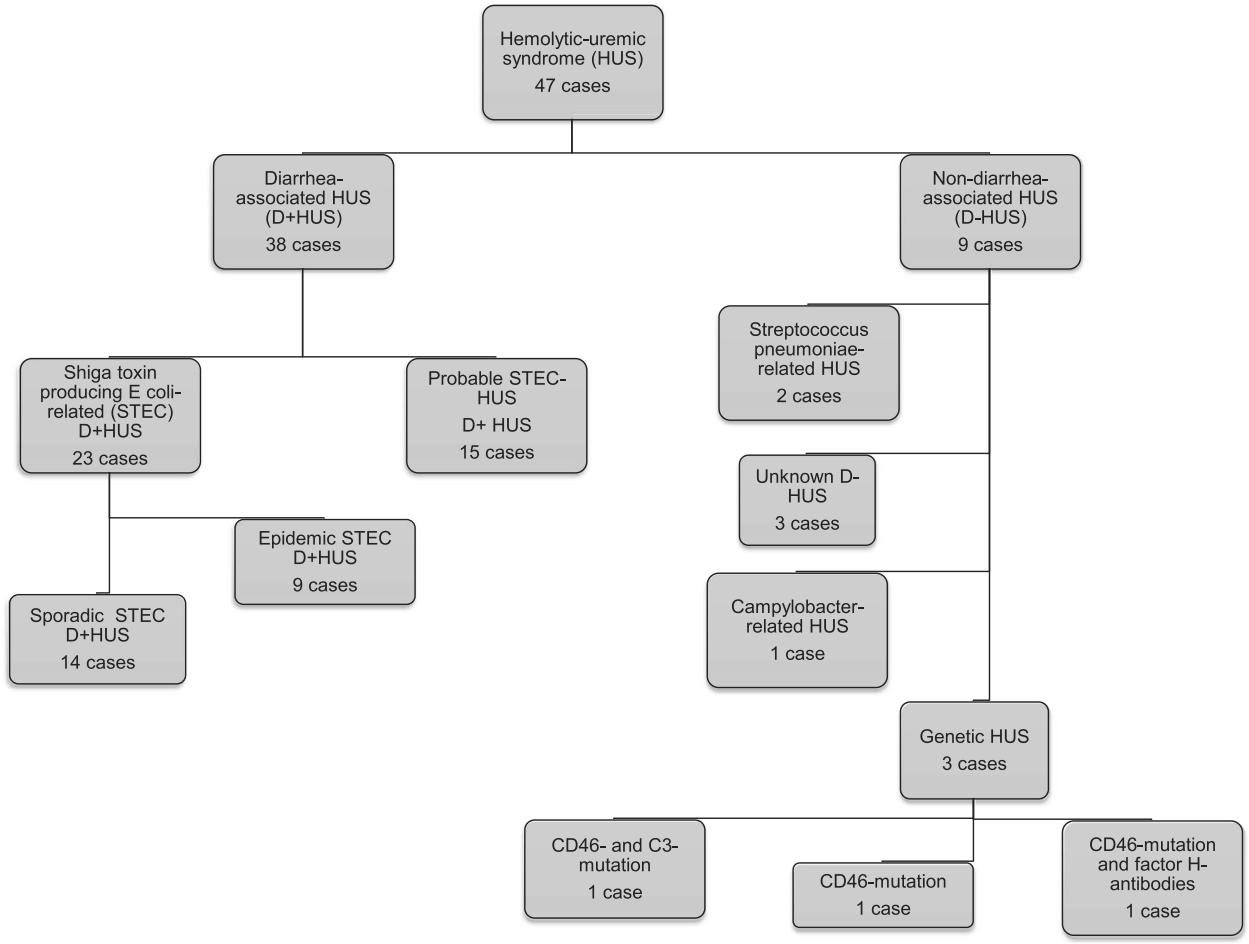

Figure 2 Etiology of HUS in children in Norway. Etiological distribution of cases of hemolytic-uremic syndrome (HUS) in children <16 years of age, Norway, 1999–2008 (n = 47).

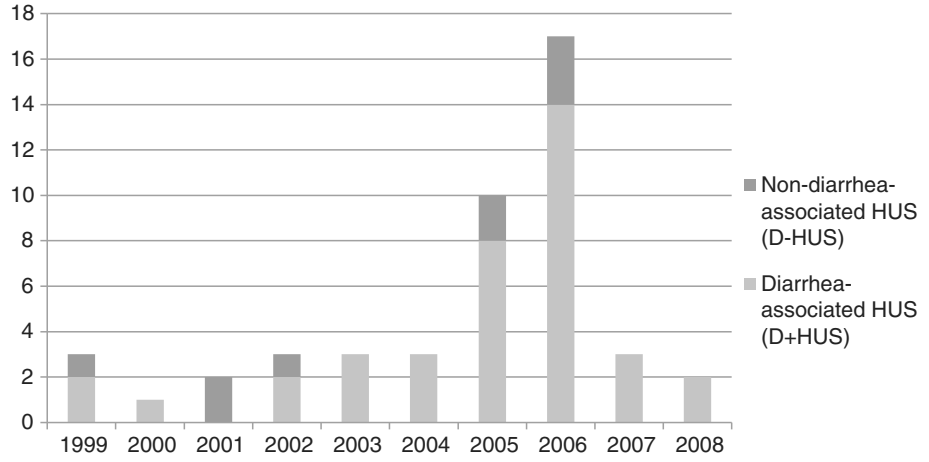

Figure 3 Yearly occurrence of HUS in children in Norway between 1999 and 2008. Yearly occurrence of cases of hemolytic-uremic syndrome (HUS) in children <16 years of age, Norway, 1999–2008 (n = 47).

Table 2 Epidemiology of HUS in children in Norway between 1999 and 2008

Type of HUS	STEC-HUS	Probable STEC-HUS	Total D+HUS			D-HUS		All HUS		
Measure	Cases (N)	Cases (N)	N	%	IR	N	%	N	%	IR
Age										
0-4 y	19	11	30	79	1.0	8	89	38	81	1.3
0 y	2	1	3	8	0.5	2	22	5	11	0.9
1 y	7	5	12	32	2.1	4	44	16	34	2.7
2 y	5	3	8	21	1.4	0	0	8	17	1.4
3 y	0	2	2	5	0.3	0	0	2	4	0.3
4 y	5	0	5	13	0.8	2	22	7	15	1.2
5-9 y	3	3	6	16	0.2	1	11	7	15	0.2
10-15 y	1	1	2	5	<0.1	0	0	2	4	<0.1
Total	23	15	38	100	0.4	9	100	47	100	0.5

Age-specific distribution (in number), proportion (in percentage) and incidence rate (IR; in average annual incidence rate in cases per 100,000 children) for diarrhea-associated (D^+HUS), with and without laboratory identified STEC infection (probable STEC-HUS) and the two combined, non-diarrhea-associated (D^-HUS) and all of the cases of hemolytic-uremic syndrome (all HUS) in children in Norway, 1999–2008 (N = 47).

Excluding nine O103 cases from the 2006 outbreak [21,22], O157 was the most common serogroup involved in sporadic D^+HUS, found in five cases (36%). The remaining nine (64%) sporadic cases were non-O157. *Stx* presence was reported in 12 (52%) of the 23 STEC-HUS cases, with *stx2* present in 10 cases and both *stx1* and *stx2* in two cases (Table 3). In the 11 cases where no *stx* was found, the strains isolated from the patients were considered STEC that had lost their toxin coding genes. Four of these strains were isolated from outbreak cases, and Multiple-locus variable-number tandem-repeats analysis (MLVA) genotyping of the strains was used to categorize these as the causative agent, even if they were *stx* negative [23].

Table 3 Serology of STEC-related HUS in children in Norway between 1999 and 2008

Case type – toxin type	Sporadic	Epidemic	Shiga-like toxin 1	Shiga-like toxin 2	Both shiga like-toxin 1 and 2
Serotype					
O26: H11	1				1
O26: H?	1			1	
O87: H?	1				
O103: H25	0	9		5	
O103: H?	2				
O145: H25	1				
O145: H?	1				
O157: H7	2			2	
O157: H?	3			2	
Non-O103/O157	2				1
Total	14	9	0	10	2

Distribution of serotype (O: cell wall antigen number, H: flagella antigen) and shiga-like toxin profile in sporadic and epidemic cases of shiga toxin producing *E. coli*-related hemolytic-uremic syndrome in children in Norway, 1999–2008 (N = 23).

D^-HUS/atypical HUS

Of the 47 identified HUS cases, 9 (19%) were considered D^-HUS. Five were male and four were female. Average annual incidence rate was <0.1 per 100,000 children (range; lowest and highest year, respectively; 0.0-0.3) (Table 2). Eight (89%) of the nine children were <5 years of age, and the last case was nine years. Two cases were related to pneumococcal infection (SP-HUS) and three were of genetic origin. All three patients had CD46-mutations. One had an additional C3-mutation and another had antibodies to factor H. In one case, *Campylobacter* was isolated and specified as causative in the medical record, without prodromal diarrhea. The remaining three were non-diarrhea-associated cases with unknown etiology (Figure 2).

Discussion

In the period 1st of January, 1999, to the 31st of December 2008, we identified a total of 47 cases of HUS in children <16 years of age, with an estimated average annual incidence rate of 0.5 cases per 100,000 children. Of these cases, 81% were diarrhea-associated HUS (D^+HUS), though only 61% of these were laboratory verified with a Shiga toxin-producing *Escherichia coli* (STEC) infection. We also found that before mandatory notification criteria were changed from D^+HUS with laboratory verified STEC infection to clinical D^+ HUS in December 2006 [18], only 61% of D^+HUS cases were notified. After the case definition was amended, the number of cases notified to MSIS corresponds with the number of cases we found when systematically reviewing all patient medical records with relevant ICD-10 codes.

We assume that all D^+HUS cases reported via MSIS are caused by STEC, since this is internationally recognized to be the most common etiological agent in HUS cases [12,18]. All D^+HUS cases in our study are therefore coded

as STEC cases. However, only 61% of the D^+HUS cases found through our review of medical records had laboratory-verified STEC. This may reflect that the HUS cases were caused by other etiological agents causing D^+HUS that we were not able to recognize, but it is more likely due to problems with diagnosing STEC in stool samples from HUS patients. HUS typically develops as a complication of STEC-related diarrhea, but patients often no longer have diarrhea when they develop HUS and may have stopped shedding the bacteria [1]. In addition, if the patient has non-O157:H7 STEC, the diagnostic methods are more complicated than if STEC is caused by O157:H7, allowing the etiological agent to be overlooked. This is especially true for cases occurring before the outbreak in 2006, as during that period many laboratories still based their diagnostics on cultivation and could easily miss the diagnosis [18]. In our study, we found that 64% of verified STEC cases were non-O157 when the outbreak-related STEC-HUS cases are excluded. However, O157 was the most frequently isolated serogroup causing sporadic HUS in Norway, as has been found in other European countries [6,7,10,11,14], South America [24,25] and North America [15,26].

Based on surveillance data from MSIS, an estimated 23% of children with STEC infections developed HUS, which is high in comparison to other countries; certain studies have shown that 10%-15% of children infected with STEC O157 develop HUS [6,12,26,27]. According to the European Center for Disease Prevention and Control, this proportion is about 8% [3]. However, these studies are based predominantly on O157 STEC cases. In the 2012 Germany outbreak, where a particularly aggressive strain of *E. coli* O104 was the cause, 22% of adults and a slightly higher proportion of children (approximately 24%) developed HUS [20]. The high proportion of HUS cases reported via MSIS may be explained by either an overestimation of HUS cases, an underreporting of STEC cases, or that STEC in Norway may be more likely to cause HUS than what is described for O157 in the literature. The first explanation is unlikely; in addition to the 23 confirmed STEC-HUS cases, we also found 15 probable STEC-HUS, all with classical clinical presentation of STEC-HUS. When considering the difficulties described for laboratory identification of non-O157:H7 STEC strains especially, it is probable that several of these are actually STEC-HUS cases. It is more likely that this high STEC-HUS/STEC-ratio is due to underreporting of STEC cases. Mild cases of STEC infection may only present with diarrhea, not requiring medical attention or submission of stool samples. Only severe cases, which are more likely to be complicated by HUS, are investigated thoroughly for a source. Isolation of STEC in D^+HUS cases is also often dependent on stool samples being examined early in the disease progression [18]. Additionally, the difficulties in identifying non-O157:H7 STEC strains may result in several cases being missed, despite samples being taken and analyzed. In Norway, most of the cases are caused by non-O157:H7 STEC, and the virulence of non-O157:H7 strains is probably variable. Some strains, like sorbitol-fermenting O157, are now considered more likely to cause HUS than O157:H7 [28], whereas others might be less likely to cause HUS.

Although the proportion of STEC cases developing HUS was high, the overall incidence rate of HUS reported in our study is low compared to similar studies from other European countries, [6-8,11,14], likely due to a low incidence of diarrhea-associated HUS, since this accounts for the majority of HUS in children (81%). This again points to the low amount of STEC cases identified in this group and the study as a whole. A possible explanation for this may be the low prevalence of STEC among ruminants in Norway. In particular, surveys in sheep and cattle have found a low prevalence of O157 [18,29-31].

Our study also describes the burden and etiology of atypical HUS in children. As there is no mandatory notification of D^-HUS cases in Norway, these cases were only identified and described after our search through medical records. Only nine D^-HUS cases were identified in the ten year period, accounting for less than 20% of the total HUS burden in children. Of these, two were related to pneumococcal infection (SP-HUS), three were of genetic origin, one had a suspected associated with campylobacter infection and three were of unknown etiology. It is noteworthy that there were only two SP-HUS cases during the ten year period, as certain studies have indicated that this is an increasing problem globally [32,33].

There are some limitations to our study. As it is retrospective, it reflects the judgments made by clinicians several years ago, when HUS was relatively uncommon. A lack of awareness could have led to misdiagnosed cases. However, to minimize this we also searched for HUS cases in medical records where patients were diagnosed as acute kidney injury. We thereby identified three HUS cases without a HUS ICD-10 code added by the clinicians, instead coded only as acute kidney injury. Failure to detect STEC in HUS patients due to late sampling and diagnostic problems in the laboratory are also limiting factors in the study, as the etiology was not found in a notable proportion of cases.

Conclusions

Our findings indicate that the occurrence of HUS, although low compared to other European countries, and STEC in Norway is higher than previously assumed. While we have no apparent explanation as to why the incidence of HUS is low, a possible contributing factor might be that the prevalence of STEC is low among ruminants, a known source of infection. The diagnostics have improved after the outbreak in 2006. Despite this, the results reinforce that clinicians should perform early

stool sampling in HUS cases where STEC infection is suspected. Therefore, our recommendation is to reinforce the mandatory notification and surveillance of both D$^+$HUS and laboratory verified STEC-infections and to further develop laboratory verification techniques of emerging non-O157 STEC serotypes.

Our study also illustrates that the proportion of laboratory verified HUS cases might be low. This highlights the need for surveillance based on clinical HUS without the need for laboratory confirmation.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
GRJ led the writing of the manuscript and performed the statistical work. All authors contributed to the writing and reviewing of the article. LV, KN, EH and GRJ participated in the methodological and structural design of the study. AB, HJB, EH and GRJ participated in the design of the clinical aspects of the study. All authors have contributed in modifying and improving the study design throughout the process. GRJ and EH performed the nationwide collecting of study data, in which AB and HJB functioned as clinical consultants. All authors read and approved of the final manuscript.

Acknowledgements
We would like to acknowledge the contributions of the clinicians and secretaries of the pediatric departments of hospitals included in the study who assisted us greatly in the process of collecting our data.
We thank Emily MacDonald for improving the English in this article.
This work was supported and funded by the Norwegian Institute of Public Health, Oslo, Norway, and the Medical Student Research Program, Faculty of Medicine, University of Oslo, Oslo, Norway.

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Received: 18 September 2013 Accepted: 17 April 2014
Published: 16 May 2014

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doi:10.1186/1471-2334-14-265
Cite this article as: Jenssen *et al.*: Incidence and etiology of hemolytic-uremic syndrome in children in Norway, 1999–2008 – a retrospective study of hospital records to assess the sensitivity of surveillance. *BMC Infectious Diseases* 2014 **14**:265.

PAPER III

Jenssen et al. BMC Infectious Diseases (2016) 16:285
DOI 10.1186/s12879-016-1627-7

BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Clinical features, therapeutic interventions and long-term aspects of hemolytic-uremic syndrome in Norwegian children: a nationwide retrospective study from 1999–2008

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Abstract

Background: Hemolytic-uremic syndrome (HUS) is a clinical triad of microangiopathic hemolytic anemia, impaired renal function and thrombocytopenia, primarily affecting pre-school-aged children. HUS can be classified into diarrhea-associated HUS (D^+HUS), usually caused by Shiga toxin-producing *Escherichia coli* (STEC), and non-diarrhea-associated HUS (D^-HUS), both with potentially serious acute and long-term complications. Few data exists on the clinical features and long-term outcome of HUS in Norway. The aim of this paper was to describe these aspects of HUS in children over a 10-year period.

Methods: We retrospectively collected data on clinical features, therapeutic interventions and long-term aspects directly from medical records of all identified HUS cases <16 years of age admitted to Norwegian pediatric departments from 1999 to 2008. Cases of D^+HUS and D^-HUS are described separately, but no comparative analyses were possible due to small numbers. Descriptive statistics are presented in proportions and median values with ranges, and/or summarized in text.

Results: Forty seven HUS cases were identified; 38 D^+HUS and nine D^-HUS. Renal complications were common; in the D^+HUS and D^-HUS group, 29/38 and 5/9 developed oligoanuria, 22/38 and 3/9 needed dialysis, with hemodialysis used most often in both groups, and plasma infusion(s) were utilized in 6/38 and 4/9 patients, respectively. Of extra-renal complications, neurological complications occurred in 9/38 and 2/9, serious gastrointestinal complications in 6/38 and 1/9, respiratory complications in 10/38 and 2/9, and sepsis in 11/38 and 3/9 cases, respectively. Cardiac complications were seen in two D^+HUS cases. In patients where data on follow up ≥1 year after admittance were available, 8/21 and 4/7 had persistent proteinuria and 5/19 and 4/5 had persistent hypertension in the D^+HUS and D^-HUS group, respectively. Two D^+HUS and one D^-HUS patient were diagnosed with chronic kidney disease and one D^+HUS patient required a renal transplantation. Two D^+HUS patients died in the acute phase (death rate; 5 %).

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Conclusions: The HUS cases had a high rate of complications and sequelae, including renal, CNS-related, cardiac, respiratory, serious gastrointestinal complications and sepsis, consistent with other studies. This underlines the importance of attention to extra-renal manifestations in the acute phase and in renal long-term follow-up of HUS patients.

Keywords: Enterohaemorrhagic *E. coli* - EHEC, Epidemiology, Haemolytic uraemic syndrome, Shiga toxin producing *E. coli* – *STEC*, clinical outcome, aHUS, SP-HUS

Background

Hemolytic-uremic syndrome (HUS) is a clinical condition characterized by the triad of impaired renal function, non-immune hemolytic anemia and thrombocytopenia, and is considered one of the most common causes of acute kidney injury (AKI) in children in Europe and the Western world [1–3]. HUS mainly affects children of pre-school age [4]. In Norway, HUS is the second most common cause of AKI in children, and has an estimated average annual incidence rate of 0.5 cases per 100,000 children [5, 6]. This is lower than in most European countries [7–9].

A common classification of HUS is by clinical presentation; associated with prodromal diarrhea (D^+HUS) or not (D^-HUS). Around 90 % of HUS cases in children are D^+HUS [7, 10]. In the Western world, most cases of D^+HUS are caused by infection with Shiga toxin producing *Escherichia coli* (STEC-HUS) [4]. According to this classification, D^-HUS mainly consists of HUS caused by *Streptococcus pneumoniae* infection (SP-HUS) and HUS associated with familiar or sporadic genetic disorders of complement regulation (atypical HUS; aHUS) [11]. This classification has some limitations. STEC-HUS is generally considered D^+HUS, although some cases may present without diarrhea [4]. Some of the aHUS cases may present with diarrhea, but are eventually classified as D^-HUS. Therefore, other classifications define HUS based on both clinical associations and causal factors [12, 13]. It has been suggested that some STEC-HUS cases, especially those with more severe outcome, are genetically predisposed aHUS cases triggered by an STEC infection [14].

D^+HUS patients usually present with signs of enteropathic infection; diarrhea, often bloody and/or watery, abdominal tenderness and more rarely low grade fever [15]. Renal affection with decreasing diuresis and subsequent oliguria and/or anuria usually follows in the estimated 10–15 % who develops HUS, although temporary renal impairment can be seen due to dehydration in STEC infections without HUS. Symptoms and complications from extrarenal involvement may occur in the acute phase; most often from the central nervous system (CNS), but also of respiratory, cardiac and gastrointestinal nature [16–18]. D^-HUS may present with various and prolonged atypical symptoms [11].

The clinical features of HUS are a consequence of microvascular lesions termed thrombotic microangiopathy (TMA). TMA mainly affects arterioles and capillaries of the kidneys and the CNS and results in impaired blood vessel flow with subsequent ischemic damage in the affected organs [4, 19].

Long-term sequelae of HUS are predominantly renal with reduced glomerular filtration rate, hypertension and/or prolonged proteinuria [4]. In a large meta-analysis, it was estimated that renal sequelae without end stage renal disease (ESRD) occurred in around 25 % and an outcome of ESRD in 3 % of D^+HUS cases [20]. The death rate is considered 3–5 %, but varies between studies [20, 21]. D^+HUS and D^-HUS are associated with similar short and long-term complications, but clinical signs of kidney dysfunction are considered more frequent in the latter. The death rate is higher in SP-HUS than D^+HUS, especially in the acute phase, although the long-term renal prognosis for this group is generally favorable [11, 22].

Treatment of HUS has until recently been supportive: fluid therapy, dialysis, plasmapheresis/plasma infusion and treatment of complications [4, 23, 24]. The emergence of eculizumab, a monoclonal C5 antibody, has now provided a proven effective treatment of genetic aHUS [25]. Eculizumab has also shown potential in the treatment of D^+HUS, and further studies are currently ongoing [26].

Knowledge on HUS in Norway has been limited. The first national outbreak of STEC-HUS in Norway, in which one child died, occurred in 2006 [27]. This outbreak led to the notification criteria of HUS being changed from STEC-HUS to all D^+HUS and brought HUS to public attention [28]. We recently published national data focusing on the epidemiological and surveillance aspects of HUS in children in Norway [5]. There, we concluded that the incidence of HUS was low compared to most European countries, but higher than previously assumed. STEC-HUS is the second most common cause of acute kidney injury in children in Norway [6], but national data on sequelae and outcomes has not been presented before. The primary aim of the current study is to describe the clinical features, therapeutic interventions and long-term aspects of the cases of D^+HUS and D^-HUS included in the epidemiological study.

Methods

Design

We performed a retrospective, descriptive study of data collected directly from relevant patient medical records.

Data collection

Potentially relevant cases were identified through medical record searches for pediatric patients < 16 years of age initially admitted to a Norwegian hospital from the 1st of January, 1999, to the 31st of December, 2008, with ICD-10 codes D59.3 (HUS), N17 (AKI) and/or N00/N01/N05 (acute nephritic syndrome/rapidly progressive nephritic syndrome/unspecified nephritic syndrome). The non-HUS ICD-10 codes were included in the search and evaluated on site to identify potentially misdiagnosed cases of HUS. Cases matching the case definitions seen below were included. Cases that had been partially treated in Norwegian pediatric departments, but had initially been admitted for HUS outside of Norway, were excluded.

All Norwegian hospitals with pediatric capacity were contacted prior to the data collection to identify relevant cases. Data were collected from relevant patient medical records from 24 pediatric departments of Norwegian hospitals; directly from 18 hospitals. Data from the remaining six hospitals were collected indirectly as they confirmed in advance having transferred all relevant patients to one of the former 18 hospitals. Medical record data missing from the six hospitals were obtained by mail. Data were collected on site by two project coworkers (medical students/authors Jenssen and Hovland), with two pediatric nephrologists (authors Bangstad and Bjerre) available for phone consultations in unclear and/or difficult cases. The coordinating center of the study was the Norwegian Institute of Public Health.

Forms made with EpiData (www.epidata.dk) were used to register data. The forms were designed through a pilot project, in which we examined the availability of relevant variables in standard medical records. The registered data were stored and encrypted according to the information security standards of the Norwegian Institute of Public Health.

Case definitions

A hemolytic-uremic syndrome (HUS) case was defined as:

- a case clinically compatible with all the following laboratory findings of
  - thrombocytopenia (<150 × 10^9/L), AND
  - anemia (Hgb < 10.5 g/dL) of hemolytic origin, with elevated serum lactate dehydrogenase (LD) (>500 U/L), AND
  - acutely reduced renal function (serum creatinine >35 μmol/L for patients < 1 years of age, > 80 μmol/L for patients 1–15 years of age), AND EITHER
    - Reported presence of fragmented red blood cells (schiztocytes) on peripheral blood smear; a sign of microangiopathic changes consistent with hemolysis and an important part of HUS pathophysiology [1], or
    - if peripheral blood smear was missing in the journal; probable clinical HUS confirmed by consulting a clinician with expertise in pediatric nephrology

A diarrhea-associated HUS (D^+HUS) case was defined as a HUS case with either:

- a clinical presentation of prodromal diarrhea, without verifiable causative etiology (probable STEC-HUS), OR
- STEC-HUS, defined as a HUS case with laboratory-verified STEC-infection

A D^-HUS case was defined as any non-diarrhea-associated HUS or HUS of verified non-STEC causality.

Variables collected

The following clinical variables were collected: time from first symptom to admittance; age at admittance; duration of initial hospitalization; duration of total time hospitalized; presence of prodromal diarrhea; presence of prodromal bloody diarrhea; presence of hypertension at admittance; development of oligoanuria, hypertension and/or proteinuria in the acute phase; extra-renal complications; death in the acute phase; laboratory values at admission and minimal/maximal value (hemoglobin, creatinine, LD, platelet count, CRP, white blood cell count, sodium).

The following therapeutic intervention variables were collected: use of dialysis, type of dialysis used, duration of dialysis; use of plasmapheresis, red blood cell transfusions, platelet transfusions, plasma infusions/exchange, antibiotics (any indication); renal transplantation performed; use of other therapeutic modalities.

The following long-term/outcome variables were collected: presence of hypertension and/or proteinuria at first follow-up and at follow up 1 year or more following initial admission; presence of renal sequelae/long-term complications; estimated glomerular filtration rate and/or creatinine value at first follow-up and at follow up 1 year or more following initial admission; death at follow up.

Statistical analysis

Descriptive statistics were calculated using Microsoft Excel and are presented as proportions and median

values with ranges. Variables are presented in tables and/or in text. Estimated glomerular filtration rate (eGFR) was estimated retrospectively using the height-independent Pottel eGFR equation [29]. Descriptive analyses for the D^+HUS and D^-HUS group were done separately, but no comparative analyses were done between the two groups due to the small number of D^-HUS cases.

Results

Patients

Forty-seven HUS patients, (16 boys and 31 girls; median age 2 years, range 5 months to 15 years) were identified from 1999 up to and including 2008. 38 (81 %) were D^+HUS cases, 23 (61 %) of which had confirmed STEC infection. Nine (19 %) were D^-HUS cases; two SP-HUS, three of verified genetic origin, one specified as *Campylobacter*-related, and three non-diarrhea-associated cases with unknown etiology. The genetic HUS cases all had CD46-mutations; one had an additional C3-mutation, another had antibodies to Factor H [5].

All but one of the D^+HUS cases presented with diarrhea and 27 (71 %) of these had bloody stools. The D^+HUS case presenting without diarrhea had confirmed STEC infection. Two (22 %) of the nine patients with D^-HUS presented with diarrhea, both had bloody stools. One initially presented with non-bloody diarrhea, clinical HUS and mild infection parameters. Bloody diarrhea was only noted after transfer to a larger hospital. There the patient developed bacteremia from a central venous catheter-related *Staphylococcus aureus* infection and had *Enterococcus faecalis* identified in a urine sample. This patient later had HUS relapses and was shown to carry both a CD46 and a C3-mutation. The other D^-HUS patient was the above mentioned were only *Campylobacter jejuni* was isolated in stool samples and specified as cause in the medical record. Both were defined as D^-HUS cases due to the etiological cause.

For the D^+HUS and D^-HUS group, respectively, median time from first registered symptom to admittance was 6 and 5 days, initial hospitalization lasted a median 15 and 16 days, whereas 29/38 (76 %) and 5/9 (56 %) cases developed oligoanuria at some point during initial admission (Table 1). Two of the D^+HUS patients died, both in the acute phase, with a death rate of 5 %. None of the D^-HUS cases had died at the point of data assessment.

Few patients had registered blood pressure at admittance. In the D^+HUS and D^-HUS group, respectively, 4/17 (24 %) and 2/6 (33 %) cases were hypertensive. However, 30/36 (83 %) D^+HUS cases and all eight D^-HUS cases where information on blood pressure was available had registered hypertension at some point during initial admission (Table 1).

Non-renal clinical features of D^+HUS patients (Table 1)

Different neurological complications were seen in nine (24 %) of 38 cases in the D^+HUS group, and manifested as follows; two patients with mild brain infarctions; one with brain microinfarctions; two patients developed brain edema; one developed brain tamponade; one had clinical meningitis; one developed intracranial hematoma following a procedure; one suffered anoxic brain damage, with brain atrophy and epilepsy. Four of the patients had seizures in the acute phase, including two without further neurological complications. In one patient, brain scanning showed lowered white matter echogenicity. Finally, one patient showed signs of CNS affection, manifested as an inability to remember certain words.

Cardiac complications were seen in two (5 %) patients; one had multiple myocardial infarctions and cardiac arrest with successful resuscitation, the other developed pericardial fluid effusion following an episode of sepsis.

Respiratory complications were described in ten (26 %) cases, with the need of ventilation therapy described in nine patients. One patient had pneumothorax. Three had hydrothorax, one of which did not receive ventilation therapy. One developed pulmonary collapse and chronic respiratory failure. The remaining patients needed ventilation therapy in the process related to other complications listed here, including sepsis and neurological events.

Five (13 %) patients had serious gastrointestinal complications. Two patients developed colonic necrosis with perforation, peritonitis and sepsis, requiring left hemicolectomy and subtotal colectomy, respectively. Two patients experienced gall stone problems, one of them requiring cholecystostomy. Other gastrointestinal complications included one patient with rectal prolapse and two with intestinal invagination. One patient in the D^+HUS group also had pancreatic complications, with the developement of diabetes mellitus.

There were eleven (29 %) cases complicated by sepsis. *Staphylococcus aureus* was specified as causative agent in two, *Staphylococcus epidermidis* in another two. One was caused by streptococcal throat infection, one by *Acinetobacter baumannii* and one had urosepsis but the agent was not specified. In the remaining cases, we were unable to identify the causative agent; two cases were complications of a perforated intestine and STEC infection proven in four without conclusive evidence of causing sepsis. Two patients developed septic shock; in both, STEC (serotype O87 and O103, respectively) was the only agent identified.

Non-renal clinical features of D^-HUS patients (Table 1)

The most severe symptoms were in the two SP-HUS patients with septicemia, neurological and respiratory

Table 1 Clinical features of HUS in children in Norway, 1999-2008

Clinical feature	Diarrhoea-associated HUS (*N* = 38)	Non-diarrhea-associated HUS (*N* = 9)
Time first symptom to admittance (median, days)	6 (4–9)	5 (2–10)
Age at admittance (median, months/years)[a]	31 (range; 5 months–15 years)[a]	18 (range; 7 months–6 years)[a]
Duration of initial hospitalization (median, days)	15 (11–24)	16 (8–42)
Duration of total time hospitalized[b] (median, days)	18 (12–24)	16 (8–53)
Prodromal diarrhea (*n*, %)	37 (97 %)	2 (22 %)
Prodromal bloody diarrhea (*n*, %)	27 (71 %)	2 (22 %)
Hypertension at admittance (*n*, %)	4 (24 %) (*N* = 17)	2 (33 %) (*N* = 6)
Hypertension registered during admittance (*n*, %)	30 (83 %) (*N* = 36)	8 (100 %) (*N* = 8)
Oligoanuria (*n*, %)	29 (76 %)	5 (56 %)
Death acute phase (*n*, %)	2 (5 %)	0 (0 %)
Non-renal complications		
Neurological complications (*n*, %)	9 (24 %)	2 (22 %)
Cardiac complications (*n*, %)	2 (5 %)	0 (0 %)
Respiratory complications (*n*, %)	10 (26 %)	2 (22 %)
Gastrointestinal complications (*n*, %)	5 (13 %)	1 (11 %)
Sepsis (*n*, %)	11 (29 %)	3 (33 %)
Renal outcome		
Proteinuria at first follow-up (*n*, %)	16 (50 %) (*N* = 32)	7 (78 %)
Proteinuria ≥ 1 year after initial admission (*n*, %)	8 (38 %) (*N* = 21)	4 (57 %) (*N* = 7)
Hypertension at first follow-up (*n*, %)	10 (31 %) (*N* = 32)	5 (56 %)
Hypertension ≥ 1 year after initial admission (*n*, %)	5 (26 %) (*N* = 19)	4 (80 %) (*N* = 5)
Chronic kidney disease (*n*, %)	2 (5 %)	1 (11 %)
End-stage renal disease (ESRD)	1 (3 %)	0 (0 %)

Results are presented as number of cases, *n* (%) and median (interquartile range). If data on the feature was not available in all medical records, the number of cases where available is presented (*N* = number of cases where available). *HUS* hemolytic uremic syndrome
[a]Range; smallest and highest value for illustrational purposes
[b]Time hospitalized including all readmissions for complications and extensive (not regular) follow-up

complications. Complications included pneumococcal-induced septic meningitis with acute respiratory failure, development of brain atrophy, hemiplegia with spastic convulsions and epileptic activity, neuronal hearing loss and retinopathy, seizures related to pneumococcal septic pneumonia with pleural empyema and acute respiratory failure. Both patients needed ventilator therapy.

The third case of sepsis in the D$^-$HUS group was of *Staphylococcus aureus* origin, after complications with a central venous catheter.

One D$^-$HUS patient developed gall-stone problems during admission, eventually needing endoscopic retrograde cholangiopancreatography-guided extraction.

Long term sequelae (Table 1)

Follow up data 1 year or more after initial admission were available in more than half the cases. With the exception of one case from 2008, all D$^+$HUS medical records were assessed at least 1 year after being diagnosed with HUS. The D$^-$HUS medical records were assessed a minimum of 2 years following primary admission.

At the first follow up after being released from hospital, presence of persistent proteinuria was seen in 16/32 (50 %) cases in the D$^+$HUS group and 7/9 (78 %) cases in the D$^-$HUS group. At follow up at 1 year or more following initial admission, presence of persistent proteinuria was seen in 8/21 (38 %) cases and 4/7 (57 %) cases, respectively. Persistent hypertension was seen in 10/32 (31 %) cases in the D$^+$HUS group and 5/9 (56 %) cases in the D$^-$HUS group at the first follow up, and in 5/19 (26 %) and 4/5 (80 %) cases at follow up 1 year or more following initial admission, respectively.

Within the time frame from initial admission to last follow up and/or registered follow up assessed in the data collection, two (5 %) of the 36 D$^+$HUS patients that survived the acute phase and one (11 %) of the D$^-$HUS patients had been diagnosed with chronic

kidney disease, and one had developed ESRD requiring renal transplantation.

Therapeutic interventions (Table 2)

Table 2 presents the therapeutic interventions implemented for all HUS patients. In the D^+HUS and D^-HUS group, dialysis was performed in 22 (58 %) and three (33 %) cases, for a median duration of 8 and 12 days, and the most common modality was hemodialysis, utilized in 16/22 (73 %) and 2/3 (66 %) cases needing dialysis, respectively. Duration of dialysis was performed at primary admission only, with one exception; one patient never recovered kidney function and continued dialysis for an additional 133 days. Plasmapheresis was performed in three (8 %) and one (11 %), plasma infusions used in six (16 %) and four (44 %) and red blood cell transfusions used in 34 (89 %) and all of the cases in the D^+HUS and D^-HUS group, respectively. Antibiotics were given in 23 (61 %) of D^+HUS cases and four (44 %) of D^-HUS cases. However, time of administration was often unclear or not specified in the medical records.

Table 2 Therapeutic interventions in HUS in children in Norway, 1999–2008

Therapeutical interventions	Diarrhoea-associated HUS ($N = 38$)	Non-diarrhea-associated HUS ($N = 9$)
Dialysis – any type (*n*, %)	22 (58 %)	3 (33 %)
Type of dialysis (n)	($N = 22$)	($N = 3$)
– Peritoneal (*n*, %)	6 (27 %)	1 (33 %)
– Hemodialysis (*n*, %)	13 (59 %)	2 (66 %)
– Both (*n*, %)	3 (14 %)	0 (0 %)
Duration of dialysis (median, days)	8 (5–15) ($N = 22$[a])	12 (7–13) ($N = 3$)
Plasmapheresis (*n*, %)	3 (8 %)	1 (11 %)
Red blood cell transfusion(s) (*n*, %)	34 (89 %)	9 (100 %)
Platelet transfusion(s) (*n*, %)	15 (39 %)	3 (33 %)
Plasma infusion(s) (*n*, %)	6 (16 %)	4 (44 %)
Antibiotics – any indication (*n*, %)	23 (61 %)	4 (44 %)
Ventilation therapy (*n*, %)	9 (24 %)	2 (22 %)
ERCP (*n*, %)	0 (0 %)	1 (11 %)
Cholecystostomy (*n*, %)	1 (3 %)	0 (0 %)
Renal transplantation (*n*, %)	1 (3 %)[b]	0 (0 %)

Results are presented as number of cases, *n* (%) and median (interquartile range). The values for type and duration of dialysis are estimated from those who received dialysis only, as specified (N = number of cases). *HUS* hemolytic uremic syndrome, *ERCP* endoscopic retrograde cholangiopancreatography
[a]Including the only patient that received dialysis after initial admission (for an additional 133 days until renal transplantation)
[b]12 months after initial admission

This also applied to indication for treatment, which included various conditions such as sepsis, catheter infection, pneumonia and urinary tract infection.

Laboratory data (Table 3)

Table 3 presents the laboratory values registered for the two groups. Notably, in the 31 patients in the D^+HUS where available, median hemoglobin value at admission was 11.1 g/dL.

Discussion

In this nationwide retrospective survey on clinical, therapeutic and long-term aspects of hemolytic-uremic syndrome (HUS) in children in Norway, we describe the multiorgan burden of this life threatening disease. A substantial amount of the patients had a complicated inhospital period and long term renal complications were common. Over a 10 year period, a total of 47 HUS cases were identified in the period [5]. D^+HUS was most common with 38 (80 %) cases, of which 23 (61 %) had confirmed STEC infection. There were nine (19 %) D^-HUS cases; two were caused by pneumococci, three were of genetic origin, one was specified as caused by campylobacter and the remaining three had unknown etiology. Because of the low number of cases and the diverse etiologies comprising the D^-HUS group, direct comparison between these groups was difficult. Similar studies exist on the HUS in other countries; with this work we have presented data on the HUS situation in Norwegian children, on which knowledge has been limited.

All but one of the confirmed STEC cases presented with diarrhea. Two of the D^-HUS cases initially presented with bloody diarrhea; these had documented atypical causes, and were classified as D^-HUS. This reflects the fact that some atypical HUS cases may present with diarrhea and emphasizes the importance of thorough diagnostic work to avoid potentially misdiagnosed cases based on early clinical presentation [4, 13]. This underlines one of the advantages of a more specific classification of HUS. Concomitantly, the relatively low frequency of these occurrences in our study also indicates that the D^+/D^- classification may be useful, especially in early etiological considerations. Another point related to initial presentation is that the median value of hemoglobin at admission was 11.1 g/dL in the 31 D^+HUS cases where available. A high level of hemoglobin at admission and even at diagnosis has also been described elsewhere [24]. This may reflect serious dehydration or that some patients were admitted before the most acute phase of hemolysis. In either case, this may be misleading in the early diagnostic work; an important point to consider when approaching a case

Table 3 Laboratory data in HUS in children in Norway, 1999–2008

Laboratory feature	Diarrhoea-associated HUS (*N* = 38)	Non-diarrhea-associated HUS (*N* = 9)
Hemoglobin at admission (median, g/dL)	11.1 (7.8–12.7) (*N* = 31)	6.7 (6.2–7.2) (*N* = 7)
Hemoglobin, minimum value (median, g/dL)	6.5 (5.8–7,5)	6.0 (5.9–6.2) (*N* = 8)
Creatinine at admission <1y (median, μmol/L)	35 (31–250) (*N* = 3)	86 (61–110) (*N* = 2)
Creatinine at admission ≥1y (median, μmol/L)	135 (61–275) (*N* = 25)	115 (110–132) (*N* = 5)
Creatinine, maximum value <1y (median, μmol/L)	231 (197–348) (*N* = 3)	97 (67–126) (*N* = 2)
Creatinine, maximum value ≥1y (median, μmol/L)	355 (200–465) (*N* = 35)	228 (124–307) (*N* = 6)
eGFR at admission <1y (median, ml/min/1,73 m^2)	42.8 (23.0–49.1) (*N* = 3)	21.8 (12.4–31.2) (*N* = 2)
eGFR at admission ≥1y (median, ml/min/1,73 m^2)	16.4 (11.0–58.5) (*N* = 25)	19.4 (18.5–28.0) (*N* = 5)
eGFR, minimum value <1y (median, ml/min/1,73 m^2)	6.5 (4.9–7.8) (*N* = 3)	21.6 (12.1–31.0) (*N* = 2)
eGFR, minimum value ≥1y (median, ml/min/1,73 m^2)	15.0 (6.3–13.8) (*N* = 35)	13.9 (7.6–21.8)
LD[a] at admission (median, U/L)	2241 (1153–2728) (*N* = 17)	2075 (1863–2659) (*N* = 5)
LD, maximum value (median, U/L)	3146 (2559–4023)	3090 (2441–5931) (*N* = 7)
Platelet count at admission (median, $\times 10^9$/L)	59 (39–175) (*N* = 30)	39 (24–107) (*N* = 7)
Platelet count, minimum value (median, $\times 10^9$/L)	32 (20–50)	24 (19–55) (*N* = 8)
CRP[b] at admission (median, mg/L)	14 (9–30) (*N* = 30)	13 (2–21) (*N* = 6)
CRP, maximum value (median, mg/L)	67 (19–138) (*N* = 37)	29 (15–161) (*N* = 7)
WBC[c] count at admission (median, $\times 10^9$/L)	17.0 (11.2–25.4) (*N* = 29)	11.6 (9.4–14.1) (*N* = 7)
WBC count, maximum value (median, $\times 10^9$/L)	19.4 (15.1–29.4)	16.0 (14.4–17.4) (*N* = 8)
Sodium at admission (median, μmol/L)	134 (130–137) (*N* = 27)	135 (130–135) (*N* = 6)

Results are presented as number of cases, *n* (%) and median (interquartile range). If data on the feature was not available in all medical records, the number of cases where available is presented with (*N* = number of cases where available). Estimated glomerular filtration rate (eGFR) was estimated retrospectively using the height-independent Pottel eGFR equation [29]. *HUS* hemolytic uremic syndrome

[a]*LD* Lactate dehydrogenase

[b]*CRP* C-reactive protein

[c]*WBC* white blood cell

with the initial clinical presentation of STEC infection and D^+HUS.

Our data on the D^+HUS group were in accordance with other studies concerning urine production at the time of admission; 76 % were oligoanuric when admitted to hospital, and 58 % required dialysis [9, 30]. Neurological complications, including seizures, brain infarction and development of epilepsy, were seen in 24 % of the cases, which is comparable to other reports [16, 31, 32]. Other neurological complications were documented, such as brain oedema and neurocognitive problems. A recent publication showed impaired neuromotor outcome in all patients included [33]. Unfortunately, we have no comparable documentation on the long term neuromotor function in this study.

Interestingly, 61 % in the D^+HUS group were treated with antibiotics prior to and/or during initial hospitalization, although for several indications. This included sepsis, which was documented in 29 %. There has been disagreement on the use of antibiotics in both STEC and STEC-HUS cases. The current consensus advices against the use of antibiotics in STEC infections because of an assumed increased risk for HUS development as a consequence of toxin release [34]. Studies have shown variable results, and the use of antibiotics depends on several factors requiring a more nuanced approach [35, 36]. The use of antibiotics in established STEC-HUS is more controversial, although studies have shown no influence on long-term outcome [34]. We were not able to examine potential effects of the use of antibiotics in our study as the time of administration was often unclear and the indication was variable.

In the D^+HUS cases where data on follow-up was available 1 year or more following initial admission, 21 % had persistent hypertension, 32 % persistent proteinuria and 8 % developed chronic kidney disease, one with need of a kidney transplant. These numbers are comparable to those described in other studies [20, 37]. The results are likely overestimated as a consequence of selective patient follow-up according to disease severity. Interestingly, previous studies have shown that some patients can develop sequelae such as hypertension and proteinuria several years after initial admission, even when showing no signs of sequelae in early follow-up [37]. This had led to the recommendations of follow up controls for at least 5 years for D^+HUS patients. The case fatality rate in

the D^+HUS group was 5 %. This rate varies between studies, and is often higher during outbreaks, but is usually considered 3–5 % [4, 21].

In the D^-HUS group, five of the nine patients were oligoanuric and of these three needed dialysis. In the study from Constantinescu [11] the renal complications of the D^-HUS group were seemingly more pronounced than we could document. However, the number of cases in our study was low, which could have influenced the results. It should also be noted that the cases included here were treated before Eculizumab was introduced as an effective treatment in genetic HUS [25].

Only two patients were diagnosed with SP-HUS. Both were severely sick in the acute phase as a complication of their pneumococcal infection. SP-HUS is generally considered more lethal in the acute phase and D^-HUS associated with more frequent long-term complications than D^+HUS [11, 22]. In our study, the long-term consequences documented in SP-HUS were sustained bilateral loss of hearing, epileptic activity and spastic hemiplegia, but none of the two patients died.

There were limitations in this study. Firstly, due to the small size of the groups and different etiologies of the D^-HUS group, comparison between them and to other studies was difficult and only descriptive results are therefore presented. Secondly, there was one outbreak of STEC leading to HUS in the 10-year period described here. This occurred in 2006 and included nine patients with STEC-HUS caused by STEC O103:H25 [27]. This outbreak seemingly had an unusually high STEC-HUS to STEC ratio, potentially caused by a particularly virulent strain. The outbreak constitute one fourth of the D^+HUS group in this study, and may have affected the results presented.

Another issue that has to be addressed is the inclusion criteria and the considerations around inclusion of some of the cases that did not strictly fulfill the criteria. Serum creatinine was chosen instead of the pRIFLE criteria because a pilot project revealed difficulties in retrospectively obtaining data on urinary output in the medical records. We did not predict the problem with such a steep rise from one to 2 years of age in these criteria. If followed categorically, this would have excluded five cases. Three D^+HUS cases and one D^-HUS case had clinical HUS and serum creatinine below 80 μmol/L, but above laboratory age-related reference level at the hospitals in question. We decided to include them as regular HUS cases. A second D^-HUS case had two admissions with reduced kidney function and falling serum hemoglobin and platelets, albeit not below our criteria. This case had an extensive family history of genetic HUS, confirmed corresponding mutations and later had recurring milder episodes. This would be considered a partial HUS case, but we decided to include it, albeit not in all estimations of clinical aspects. A sixth patient died early in the acute phase, with s-hemoglobin value only documented at admission and higher than required in our criteria. This patient had confirmed STEC infection. These cases were included after consulting a clinician with expertise in pediatric nephrology.

These challenges highlight some important limitations to these types of studies, especially when evaluating an extensive amount of data. There are two important factors in particular that need to be commented. One; we designed the data collection form from a pilot study of HUS medical records to assess which parameters where both relevant and available. Two; data collected from medical records were subject to the standards of different hospitals, clinicians and the subjective (and objective) opinions of the latter. Certain parameters were generally fixed and difficult to misinterpret, others were not. For example; "duration of initial hospitalization" was not subject to misinterpretation as the dates followed the medical records. On the other hand, "time from first symptom to admittance" was subject to uncertainty according to how clinicians had perceived disease progression (e.g. "a couple of days" or similar).

Another limitation is the consistency in a retrospective survey of medical records to provide all necessary data. All medical records and associated charts were examined thoroughly. However, we were only able to obtain measures of blood pressure at admittance in 23 cases. This only allowed us to assess blood pressure at admittance in less than half the cases, illustrating the potential for missing data in retrospective surveys.

Conclusions

We have presented the clinical features, therapeutic interventions and long-term aspects of hemolytic-uremic syndrome in children in Norway over a 10-year period. A nationwide collection of data has allowed us to include all cases that occurred within this time span, describing this life-threatening condition on which knowledge concerning disease burden and outcome was limited. The data reports on the multi-organ affection in this disease entity with a high numbers of serious complications. These include a considerable number of cases with severe complications from the central nervous system, with brain micro infarctions and edema and development of epilepsy, of cardiac nature, such as myocardial infarction, in the gastrointestinal tract, such as colonic perforation and subsequent peritonitis, the respiratory system, such as acute respiratory failure, and a large proportion developing sepsis in the acute phase. These data underline that HUS patients have to be

monitored carefully for extra-renal involvement in the acute phase. There were also a considerable number of cases showing long-term kidney related sequelae. Children with symptoms suspicious of HUS should be treated at centers with experience and possibilities for thorough monitoring. Through this and previous studies, we would like to emphasize the importance of thorough long-term follow-up and the need for quality guidelines to ensure this aspect of patient care in patients affected by HUS.

Abbreviations

aHUS, atypical HUS; AKI, acute kidney injury; CNS, central nervous system; D^+HUS, diarrhea-associated HUS; D^-HUS, non-diarrhea-associated HUS; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HUS, hemolytic-uremic syndrome; LD, lactate dehydrogenase; SP-HUS, *Streptococcus pneumoniae*-associated HUS; STEC, Shiga toxin producing *Escherichia coli;* TMA, thrombotic microangiopathy

Acknowledgements

We would like to acknowledge the contributions of the clinicians and secretaries of the pediatric departments of hospitals included in the study who assisted us greatly in the process of collecting our data.

Funding

This work was supported and funded by the Norwegian Institute of Public Health, Oslo, Norway, the Medical Student Research Program, Faculty of Medicine, University of Oslo, Oslo, Norway and the Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway.

Availability of data and materials

We were granted exemption from patient confidentiality regulations that require informed consent to access patient medical records. One of the conditions for this was that any associated publications would not present data in a way that could be potentially person identifiable. Because of this, our data are to the highest possible degree summarized, not individualized. In the datasets, information is listed on an individual basis (albeit without directly personally identifiable information, such as name, personal number, address). As some of our cases were subject to media attention, making the dataset publicly available would create the possibility of cases being recognized. Therefore, in accordance with the granted exemption, the dataset will not be made public.

Authors' contributions

GRJ wrote the manuscript draft, led the writing process and performed the statistical work. All authors contributed to the writing and reviewing of the article. LV, KN, EH and GRJ participated in the methodological and structural design of the study. AB, HJB, EH and GRJ participated in the design of the clinical aspects of the study. All authors have contributed in modifying and improving the study design throughout the process. GRJ and EH performed the nationwide collecting of study data, in which AB and HJB functioned as clinical consultants. All authors read and approved of the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

This manuscript does not contain directly personally identifiable information. In addition, as stated above, the parents were notified with the option to ask for exclusion of all data from their child's medical records from the study. When notified, they were also informed that the results of this study would be published. None of the parents chose to exclude data from the study.

Ethics approval and consent to participate

The Regional Ethics Committee South East A approved this study. The Norwegian Directorate of Health granted us exemption from patient confidentiality regulations that require informed consent to access patient medical records. This was necessary as potential cases would only be identifiable following review of medical records. When cases were identified, the parents were notified with the option to ask for exclusion of all data from their child's medical records from the study. If the parents chose not to respond, the data from hospitalisations of their child was included in the study. None of the parents chose to exclude data from the study.

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Received: 31 January 2016 Accepted: 7 June 2016
Published online: 13 June 2016

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PAPER IV

ACTA PÆDIATRICA
NURTURING THE CHILD

Acta Pædiatrica ISSN 0803-5253

REGULAR ARTICLE

The incidence and aetiology of acute kidney injury in children in Norway between 1999 and 2008

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Keywords
Acute kidney injury, Aetiology, Epidemiology, Haemolytic-uraemic syndrome, Nephritic syndrome

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Received
13 March 2014; revised 18 May 2014; accepted 8 July 2014.

DOI:10.1111/apa.12742

The copyright line for this article was changed on 7 May 2015 after original online publication.

ABSTRACT

Aim: Primary acute kidney injury (AKI) is a direct cause of hospitalisation in children, but can also result from other conditions. There is limited information on the epidemiology of this condition. Our aim was to describe the national incidence rate and aetiology of acute kidney injury in children under the age of 16 in Norway from 1999 to 2008.

Methods: We carried out a retrospective study of medical records provided by all 18 of the paediatric hospital departments that specialise in treating paediatric patients with AKI.

Results: We identified 315 cases of AKI (53% male), with an estimated average annual incidence rate of 3.3 cases per 100 000 children and a median annual occurrence of 33 cases. Most cases (43%) were in children under five. We identified 53 aetiologies and classified these into 30 aetiological groups: 24% of the cases were prerenal (n = 75), 74% were intrinsic/renal (n = 234) and 2% were postrenal (n = 5). Nephritic syndromes was the major cause (44%) of AKI, followed by haemolytic-uraemic syndrome (HUS) (15%).

Conclusion: Nephritic syndromes and HUS are the most common aetiologies of AKI in Norway. Although our results could indicate a low incidence of paediatric AKI in Norway, the lack of other national studies makes comparisons difficult.

BACKGROUND

Acute kidney injury (AKI), previously referred to as acute renal failure, is defined as a sudden decline in kidney function, with falling glomerular filtration rate and the inability to regulate the acid, electrolyte balance and to excrete waste and fluid (1). It is an important contributor to mortality and morbidity in paediatric patients with critical illnesses and may also be associated with mortality in mild cases of kidney failure (2). AKI can be divided into three categories, prerenal, intrinsic/renal and postrenal, depending on the pathophysiological mechanism leading to the decline in function. Some cases are difficult to categorise, due to the complex nature of different underlying conditions.

The main causes of AKI in Africa and Asia are of prerenal origin, due to dehydration, which is often caused by gastroenteritis and infections. However, with improving living conditions, the pattern is also changing in these countries (3–6). In Europe, the most common cause of AKI is haemolytic-uraemic syndrome (HUS), a clinical syndrome characterised by the triad of thrombocytopenia, microangiopathic haemolytic anaemia and acute oliguric or anuric renal failure, often characterised as either diarrhoea-associated or non-diarrhoea-associated, also called atypical, HUS (7). Outbreaks of HUS, particularly the large Northern European outbreak originating in Germany in 2011 (8), and a national outbreak in Norway in 2006 (9,10), have increased public awareness of the condition in recent years.

To our knowledge, no national reports or nationwide studies are available on the incidence of AKI, as they are usually performed at specific centres or regions, based on limited surveillance networks and registries and focusing on the incidence in hospital populations (11,12). There is an

Abbreviations
AKI, acute kidney injury; HUS, haemolytic-uraemic syndrome.

Key notes

- This study examined the national incidence rate and aetiology of paediatric acute kidney injury (AKI), with data from all 18 Norwegian paediatric hospital departments that provide specialist AKI treatment.
- Most cases (43%) were in children under five, and the major causes were nephritic syndromes (44%) and haemolytic-uraemic syndrome (15%).
- The incidence rate seemed low, at 3.3 cases per 100 000, but the lack of other national studies make comparisons difficult.

increasing interest in national registries on different diagnoses, but so far, no national registries on AKI in children exist, in contrast to registries on dialysis and transplantation. National data on AKI does not exist in Norway, but it was possible to collect data on a national basis, as the country has five million inhabitants and only a limited number of hospitals treat children with AKI.

We recognise the need to describe the importance and burden of AKI in a national context, as it plays a key role in the mortality and morbidity in paediatric patients and such data are not currently available. The incidence of HUS is relatively low in Norway (13), despite being recognised as the most common cause of AKI in Europe. We wanted to compare the incidence of AKI to other countries to see whether Norway's rate was, in fact, lower than other countries or whether the incidence rates reflected that HUS is a less important contributor to AKI in Norway. Therefore, the aim of this study was to estimate the incidence of AKI in children in Norway and describe the epidemiology. We also wanted to determine the distribution of different aetiologies of the condition.

METHODS

This was a retrospective, descriptive study, based on data from patient medical records. Potential cases were identified by searching the medical record archives at the Norwegian paediatric departments that specialise in treating children with AKI. We gathered data directly from 18 of the country's 24 paediatric departments, having confirmed that the remaining six used these centres as secondary or tertiary hospitals and referred children with AKI to them.

Search criteria were ICD-10 codes N17 (AKI), D59.3 (HUS), N00 (acute nephritic syndrome), N01 (rapidly progressive nephritic syndrome) and N05 (unspecified nephritic syndrome).

We included children who were under the age of 16 years when they were first admitted to a Norwegian hospital between 1 January 1999 and 31 December 2008. AKI cases were defined as: (i) a primary or secondary initial diagnose of acute kidney injury (ICD-10: N17) and/or haemolytic-uraemic syndrome (ICD-10: D59.3), and/or acute nephritic syndrome / rapidly progressive nephritic syndrome / unspecified nephritic syndrome (ICD-10: N00/N01/N05) plus (ii) a confirmed history with a serum creatinine increase of >35 μmol/L if the patients were under the age of one or >80 μmol/L if they were aged 1–15 years.

A HUS case was defined as a case with a clinical picture that included all of the following: (i) thrombocytopenia, with a low platelet count (<150 × 10^9/L), (ii) anaemia (Hgb <10.5 g/dL) of haemolytic origin, with increased serum lactate dehydrogenase (>500 U/L) and (iii) acutely reduced renal function, with serum creatinine of >35 μmol/L if the patient was under the age of one or >80 μmol/L if the patient was 1–15 years. To be included, the patients also had to have either: (i) a reported presence of fragmented red blood cells (schistocytes) on peripheral blood smear, a sign of microangiopathic changes consistent with haemolysis and an important part of HUS pathophysiology (14), or if peripheral blood smear was missing from their records, a probable clinical diagnosis of HUS confirmed by consulting a clinician with expertise in paediatric nephrology.

In cases with multiple admittances and/or, occurrences of AKI, only the initial episode was included. Exclusion criteria were AKI related to birth asphyxia or acute post-kidney-transplantation graft failure.

Statistical analysis

Calculations were performed using Microsoft Excel. Descriptive statistics are presented as proportions, median and annual average values with ranges and as incidence rates calculated using population numbers provided by official Statistics Norway registries.

Ethical considerations

This study was approved by the Regional Ethical Committee South East A. The Norwegian Ministry of Health granted us exemption from patient confidentiality regulations requiring informed consent to access patient medical records.

RESULTS

During the 1999 to 2008 study period, we identified 315 cases of AKI in children in Norway under the age of 16. Of these, 167 (53%) were male (Fig. 1). The estimated average annual AKI incidence rate was 3.3 cases per 100 000 children, ranging from 1.8 in the lowest year to 5.2 in the highest year. The median annual occurrence of AKI was 33 cases. The highest occurrence was in 2006, with 50 cases (16% of the total study) and the lowest was in 2000, with 17 (5%) cases (Fig. 2).

The median age at occurrence was 6 years for both male and female patients, with a range of zero to 15 years. The age-related distribution by gender can be seen in Fig. 1.

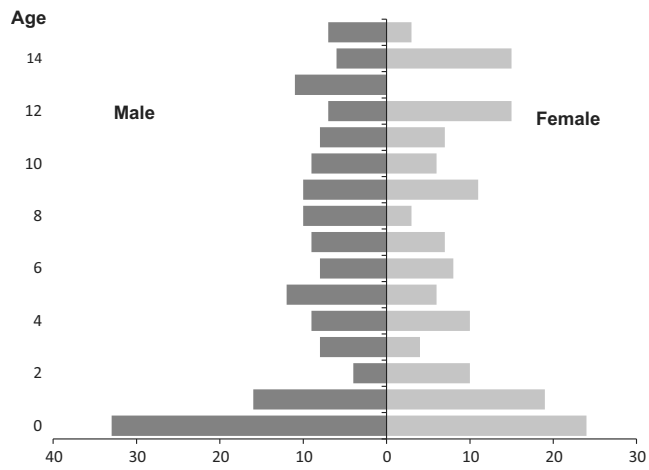

Figure 1 Distribution of cases of acute kidney injury by age and gender in children under 16 years of age in Norway, 1999–2008 (N = 315).

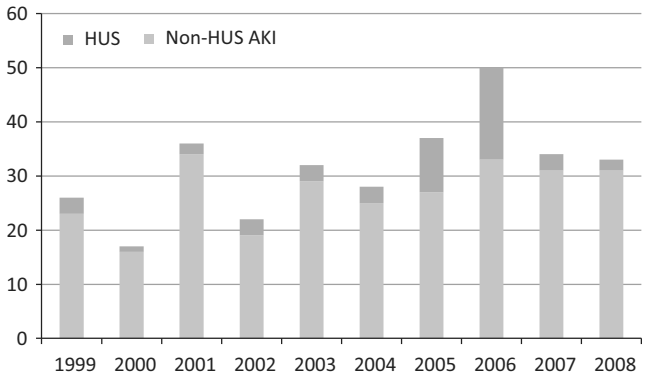

Figure 2 Yearly occurrence of acute kidney injury (AKI) and share of cases caused by haemolytic-uraemic syndrome (HUS) in children under 16 years of age in Norway, 1999–2008 (N = 315).

The highest percentage of cases was found in patients under the age of five (43%), and this age group had an estimated average annual incidence rate of 4.7 cases per 100 000 children, ranging from 1.7 to 6.9 (Table 1).

When we divided the cases into probable pathophysiological causes of kidney failure, the 315 AKI cases were distributed as follows: 24% prerenal (n = 75), 74% intrinsic/renal (n = 234) and 2% postrenal (n = 5). One case had an unknown cause.

A total of 53 different aetiologies were identified and classified into 30 different aetiological groups (Table 2), with nephritic syndromes accounting for 138 (44%) cases. It is notable that 71 of these nephritis syndrome cases occurred in patients who did not have an ICD-10 AKI diagnosis, just an episode of marked serum creatinine increase.

A further 47 (15%) AKI cases were related to haemolytic-uraemic syndrome (HUS). Of these, 9 (3%) were atypical, 38 (12%) were associated to diarrhoea. Only 24 (51%) of the HUS cases had an ICD-10 AKI diagnosis code registered in their medical record. There was a link between HUS cases and the year with the highest occurrence of AKI (Fig. 2).

Apart from HUS and nephritic syndromes, the most frequent causes identified were the 24 (8%) cases with prerenal causes in relation to septicaemia and the 23 (7%) cases of dehydration that were specified as the cause in the medical record but were either related or not related to other conditions. Table 2 presents an overview of identified aetiologies.

Table 1 Age-related occurrence, percentage and incidence rate of acute kidney injury in children in Norway, 1999–2008 (N = 315)

Measures			
Age group	Cases	Percentage of total, %	Average annual incidence rate per 100 000 children (range)
0–4 years	137	43	4.7 (1.7–6.9)
5–9 years	84	27	2.7 (1.6–5.6)
10–15 years	94	30	2.6 (2.0–3.7)
Total	315	100	3.3 (1.8–5.2)

DISCUSSION

We identified 315 cases of AKI in children during the period 1999–2008, with an estimated overall average annual incidence rate of 3.3 cases per 100 000 children. The highest incidence was found in patients under the age of five, with 137 (43%) cases and an estimated average annual incidence rate of 4.7. The year with the highest occurrence of AKI occurred in 2006 and coincided with the highest HUS occurrence (Fig. 2) (13), related to the Norwegian outbreak (9,10). We also found that the most common type of AKI was of an intrinsic/renal nature most commonly related to nephritic syndromes, followed by HUS.

In our study, we present data from patients from all 18 Norwegian hospitals capable of managing AKI. This is, to our knowledge, the first national study on the epidemiological aspects and aetiology of AKI in children in Europe. Most published studies are confined to intensive care units, tertiary centres or specific regions of a country (11,12,15). In our opinion, describing the epidemiological aspects of this serious condition makes an important contribution to evaluating the national relevance and burden of AKI.

The lack of similar national studies makes it difficult to compare our findings with other countries and state whether our incidence is high or low. However, the annual incidence rate and occurrence of cases seemed to slowly increase during the ten-year study period (Fig. 2) and this trend might reflect the suggestion in certain published papers that paediatric AKI is increasing (1). We should point out that this finding should not be considered conclusive, as we did not carry out a statistical analysis to confirm a significant increase adjusted for potential population growth. A relatively stable, yet generally increasing number of cases can be seen, with the exception of 2006, when there was a national outbreak of *Escherichia coli* O103:H25 and nine children developed HUS (9,10).

Nearly half of the cases in our study were under the age of five, and this may reflect the fact that small children are more vulnerable to gastroenteritis and other infections and more likely to suffer from dehydration and volume depletion.

With regard to the aetiological findings, nephritic syndromes was a notable and very comprehensive aetiological group and provided the most common cause of AKI, which agrees with some studies (11,12,16), and contradicts others (15,17). Of the 138 cases in this group, 71 did not have an ICD-10 code that specified the occurrence of AKI. However, 67 (21% of all AKI) cases were specified as AKI. It is therefore justifiable to consider that this was the most common cause of AKI in children in Norway.

Table 2 Distribution of aetiologies in number and percentage of cases in acute kidney injury in children in Norway, 1999–2008 (n = 315)

Prerenal			Renal			Postrenal		
Aetiological group	N	%	Aetiological group	N	%	Aetiological group	N	%
Sepsis	24	7.6	Nephritic syndromes	138	43.8	Congenital anomalies of the kidney and urinary tract	3	1.0
Dehydration	23	7.3	Haemolytic-uraemic syndrome	47	14.9	Vesicoureteral reflux	1	0.3
Cardiological aetiologies	11	3.5	Oncological	16	5.1	Pelvic tumour	1	0.3
Medical/surgical complications	5	1.6	Drug related	8	2.5			
Systemic shock	2	0.6	Congenital anomalies of the kidney and urinary tract	7	2.2			
Drowning (multiple organ failure)	2	0.6	Genetic disorders	5	1.6			
Meningitis	2	0.6	Rhabdomyolysis	5	1.6			
Acute on chronic	1	0.3	Nephropathia epidemica	2	0.6			
Appendicitis	1	0.3	Unknown renal	2	0.6			
Encephalitis	1	0.3	Severe combined immunodeficiency	1	0.3			
Hypophyseal defect	1	0.3	Intoxication	1	0.3			
Diabetes complications	1	0.3	Wegeners granulomatosis	1	0.3			
Respiratory failure	1	0.3	Cerebral palsy complications	1	0.3			
Total prerenal	75	23.8	Total renal	234	74.2	Total postrenal	5	1.6
Unknown	1	0.3						

Discovering that HUS and septicaemia leading to prerenal AKI were two of the most common causes of AKI was not unexpected and is in line with the findings of studies from other countries with comparable conditions, notably other European countries (11,12,16).

Most larger studies that compare the incidence and aetiology in a population are based on single centres and/or hospitals and are often based on children who need renal replacement. However, our study describes a national distribution of aetiologies and is based on the presence of AKI regardless of required therapeutic measurements. This means that our research covers a wider geographic and aetiological area than existing studies.

One of the advantages of carrying out a national study of AKI incidence based on medical records was the possibility of identifying all cases admitted during the study period. Another possible data source would have been the National Patient Register, but this did not contain identifiable data by patient before 2008 and we would have been limited to the number of consultations and not the number of patients diagnosed. This issue was avoided by systematically gathering data from all Norwegian hospitals capable of treating children with AKI.

There are certain limitations to this study. First, our data were retrospectively collected from medical records and, in many cases, it was difficult to identify an aetiology because complicated medical conditions meant that there were many possible factors that could have led to renal insufficiency. This meant that we had to assume that the clinician in charge of the patient had identified the main cause of the AKI. One of the prime examples of this was the aetiological group called hypovolaemia, which in theory can cause renal insufficiency as a complication of a wide range of medical conditions. We had to separate cases caused primarily by fluid loss and/or low fluid intake from those caused by pathophysiological changes from an underlying condition that in itself caused the hypovolaemic state.

Another limitation was that the number of AKI cases was probably underestimated. Only 51% of HUS cases had an ICD-10 code for AKI (N17) in their medical records, which suggests that the diagnosis code is often left out of medical records where AKI is part of a more prominent and/or severe clinical diagnosis or where it is included in a clinical syndrome with a separate ICD-10 code. As we had to limit our search to the usual ICD-10 codes for AKI, some of these cases would not have been picked up by our search criteria and would not have been included in our study.

The underestimation of AKI cases was partly reduced by including nephritic syndrome cases with marked serum creatinine increase, without the N17 diagnosis code. Although a few of these cases had miscoded ICD-10 diagnoses and were originally meant to be registered as AKI, most of them did not have sufficiently grave renal insufficiency at the time of admission. They were included in our study as they matched our inclusion criteria. However, it must be noted that the decision to include nephritic syndrome was made after we determined which of

the hospitals should be approached for direct data. Therefore, these cases were identified in the 18 hospitals where direct data were collected for this study. Although it is highly unlikely, it is possible that there were missing nephritic syndrome cases, potentially matching our inclusion criteria, in the other six hospitals with paediatric departments who referred their patients to the 18 hospitals involved in the study. Another potential problem with including the nephritic syndrome cases was that it could have overestimated the role that nephritic syndrome plays in the aetiology of AKI. However, there were two subgroups of nephritic syndrome cases that we included in our study: one in which AKI was diagnosed and the cases were tagged with an appropriate AKI ICD-10 code and another where the cases had experienced a clear rise in serum creatinine above reference values, including some that were directly described as AKI in the medical records, but were not tagged as AKI. While this may have led to the total number of nephritic syndrome cases being high, or overestimated, they are still clearly the largest group and thus the most common cause of AKI in children in Norway.

Some studies have reported that the major cause of AKI is prerenal (18), but our retrospective study revealed that most cases were renal. This could be because temporary rises in creatinine in children without septicaemia and dehydration/hypovolaemia, who do not need dialysis, are not reported, even if measures such as fluid resuscitation were performed. In addition, dehydration as a result of gastroenteritis is not a common cause of AKI in Norway.

Our decision about how to define kidney injury was based on a pilot project where data were collected to adjust the included parameters. This was performed as difficulties with collecting data on a retrospective method were probable. The pRIFLE criteria were introduced in 2004, to measure paediatric risk, injury, failure, loss and end-stage renal disease, and are based on both creatinine levels and urinary output. They define the risk of kidney injury as a 25% decrease of estimated creatinine clearance, injury as a 50% decrease and failure as a 75% decrease (19). However, our pilot project revealed obtaining data on urinary output was difficult, as they were often absent or incomplete. Thus, a creatinine level of 35 in children below 1 year of age would not overestimate the condition and a level of 80 after 1 year of age would include all.

CONCLUSION

This study estimated the incidence of acute kidney injury (AKI) in children in Norway and determined the distribution of the aetiologies involved in the development of the condition. Our study has some obvious limitations, particularly due to the retrospective design of the study, resulting in a probable underestimation of the number of AKI cases. In the future, the focus needs to be on better registration of children with AKI. The pRIFLE criteria have now been implemented and provide a standard for all paediatric departments, which should lead to better diagnosis and improved possibilities for long-term follow-up. Prospective studies are needed to provide accurate data and evaluate the outcome of AKI. HUS is considered to be the most common cause of AKI in Europe, but this was not the case in our study. Although HUS did constitute a large proportion of our cases, nephritic syndrome was the most common cause of AKI in Norway.

Throughout this study, we were also able to assess the limitations involved in this type of national epidemiological study. We hope that our observations may prove useful in future studies with similar designs and aims.

ACKNOWLEDGEMENTS

This work was funded by the Norwegian Institute of Public Health, Oslo, Norway, and the Medical Student Research Program, Faculty of Medicine, University of Oslo, Oslo, Norway. We would like to thank the clinicians and secretaries from the paediatric departments of the hospitals who took part in the study for their help in the data collection. The authors declare they have no competing interests.

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