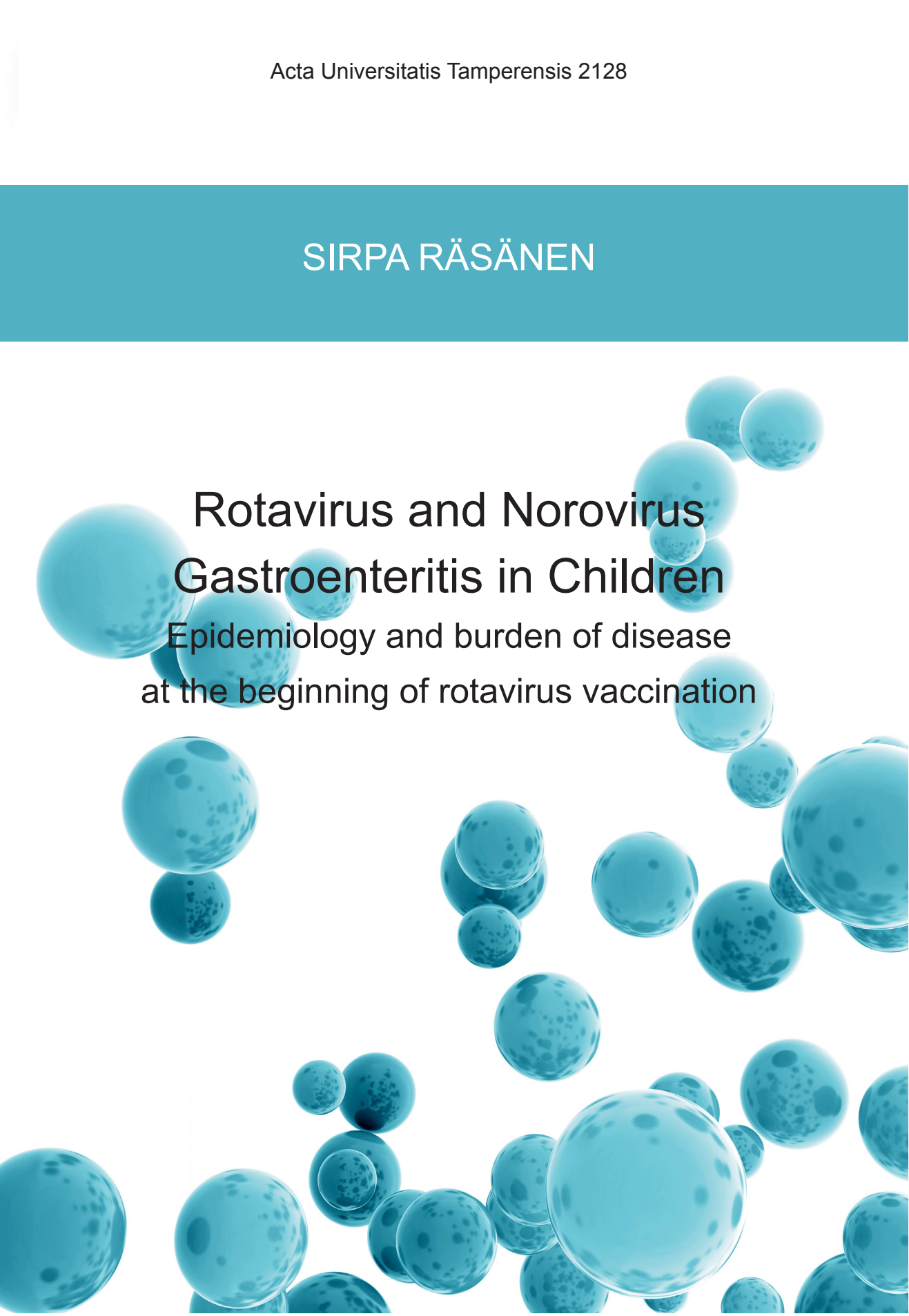SIRPA RÄSÄNEN

Rotavirus and Norovirus Gastroenteritis in Children

Epidemiology and burden of disease
at the beginning of rotavirus vaccination

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UNIVERSITY OF TAMPERE

SIRPA RÄSÄNEN

Rotavirus and Norovirus Gastroenteritis in Children

Epidemiology and burden of disease
at the beginning of rotavirus vaccination

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Abstract

Most cases of acute gastroenteritis in children in developed countries are caused by viruses. Rotaviruses were the most important causative agents of acute gastroenteritis in young children until the launch of the universal rotavirus vaccination in Finland. Noroviruses are second to rotaviruses as causative agents of acute gastroenteritis in children, and their importance is increasing after the elimination of most rotavirus gastroenteritis by vaccinations.

We studied the causative agents, epidemiology, clinical picture and severity of acute gastroenteritis in children seen at Tampere University Hospital between September 2006 and August 2008. All children presenting with acute gastroenteritis at the hospital, either in the outpatient clinic or in the hospital ward, and children who caught gastroenteritis while being treated at the hospital for another reason were eligible for enrollment. Altogether, 1193 patients were recruited, and 809 had adequate stool samples for viral studies. Stool samples were studied for viruses with RT-PCR, and also by ELISA for rotaviruses. The clinical information was obtained by interviewing the parents and from the hospital records. The parents of the children who were hospitalized were also asked to participate in a survey on the costs of acute gastroenteritis. This epidemiological study formed the baseline for the study on the first impact of universal rotavirus immunization, introduced in September 2009.

The dissertation consists of four original studies. Study **I** describes children who had acute gastroenteritis in a waterborne acute gastroenteritis outbreak in Pirkanmaa in 2007. The drinking water of about 12 000 people was heavily contaminated with sewage water, causing severe and mixed infections with several enteritis pathogens. Altogether, 115 children in this outbreak were seen at the hospital, and 65 of them were enrolled in the study. The majority of these children had severe gastroenteritis with multiple pathogens in stools. Their heavy exposure to enteropathogenic viruses and bacteria caused severe symptoms of acute gastroenteritis regardless of the causative agent(s).

Studies **II, III** and **IV** are prospective studies. In Studies **II** and **III**, two acute gastroenteritis seasons were studied to describe the burden and epidemiology of rotavirus and norovirus in acute gastroenteritis in children. In the first follow-up

season, 2006–2007, the share of rotaviruses (38%) among acute gastroenteritis cases seen at the hospital was smaller than usual, and the role of noroviruses (34%) was pronounced. In this low rotavirus season, the emerging rotavirus genotype G9P[8] was predominant, along with the traditionally dominating genotype G1P[8]. The second season, 2007–2008, was more "typical," with 62% of cases of acute gastroenteritis caused by rotaviruses and G1P[8] as the predominant genotype. In both follow-up years, a clear seasonality in rotavirus gastroenteritis was seen.

At the beginning of our study, two rotavirus vaccines had just become available. The first vaccine on the market was a human G1P[8] vaccine (Rotarix®), and the second vaccine (Rotateq®), which contains G1-4 and P[8] bovine-human reassortant rotaviruses, became available during the follow-up. The vaccine coverage of Rotarix® reached 29% and that of Rotateq® 6% by the end of the follow up in 2008; altogether the rotavirus vaccine coverage reached 35% among the target population. Such vaccination coverage may have reduced the rotavirus gastroenteritis seen at the hospital in the study period through direct efficacy and break of transmission.

The major norovirus genotype in the study seasons was GII.4. This finding is consistent with other results around the world in the 2000s. The seasonality of norovirus gastroenteritis in children is similar to that of rotaviruses, but the timing of the winter season is different. Using the 20-point severity score with a cut-off of 11/20, 31% of the RV episodes and 18% of NoV episodes were classified as severe.

We also evaluated the costs of rotavirus and norovirus gastroenteritis for society and families and described them below, in the Results section of the thesis. Extrapolating our results to the whole of Finland, we estimate that the annual medical costs for norovirus gastroenteritis at hospital level were about € 1 801 000 and those for rotaviruses gastroenteritis about € 3 714 460, evaluated in terms of 2007 prices.

Study **IV** describes the sharp decline, which was observed in rotavirus gastroenteritis in 2009–2011 after introduction of rotavirus vaccination in the national immunization programme in Finland. As predicted, after rotavirus gastroenteritis was largely eliminated by universal rotavirus vaccination, noroviruses became the most important causative agent of acute gastroenteritis in children. Norovirus gastroenteritis is sufficiently common and severe to warrant vaccination of infants or young children once a norovirus vaccine becomes available. Norovirus is a current target for vaccine development.

Tiivistelmä

Suurin osa lasten äkillisistä ripuli- ja oksennustaudeista (akuutti gastroenteriitti) teollisuusmaissa on virusten aiheuttamia. Suomessa rotavirukset olivat pitkään merkittävimpiä pikkulasten ripulitaudin aiheuttajia siihen asti kun lasten rokotusohjelmaan otettiin vuonna 2009 rotavirusrokote. Norovirukset ovat olleet toisella sijalla lasten äkillisen ripuli- ja oksennustaudin aiheuttajana, ja niiden merkitys on edelleen korostunut kun rotavirusgastroenteriitit ovat vähentyneet rokotusten ansiosta.

Väitöskirjatyön tarkoituksena oli selvittää erikoissairaanhoidon arviota vaatineiden ripuli-oksennustautien aiheuttajia, epidemiologiaa ja kliinisten tautien oirekuvaa ja vakavuutta lapsilla. Seuranta tehtiin Tampereen yliopistosairaalan (Tays) lastenklinikassa 1.9.2006 – 31.8.2008. Tutkimukseen pyrittiin saamaan mukaan kaikki lapset, jotka tulivat lasten päivystyspoliklinikalle ja/tai lasten infektio-osastolle akuutin gastroenteriitin vuoksi, tai jotka sairastuivat gastroenteriittiin ollessaan sairaalassa muusta syystä. Yhteensä tutkimukseen osallistui 1193 potilasta, joista 809:lta saatiin ulostenäyte tutkimuksiin. Ulostenäytteistä tutkittiin ripulitautia aiheuttavat virukset RT-PCR -menetelmällä ja rotavirusten osalta myös ELISA-menetelmällä. Sairaustiedot saatiin haastattelemalla vanhempia ja sairaalan potilaskertomuksista. Sairaalassa osastohoidossa olevien lasten vanhempia pyydettiin myös mukaan gastroenteriitin taloudellisia seuraamuksia kartoittavaan tutkimukseen.

Tämä epidemiologinen tutkimus toimii vertailutietona tutkittaessa syyskuussa 2009 yleiseen rokotusohjelmaan otetun rotavirusrokotteen varhaisvaiheen vaikutuksia lapsiväestössä.

Väitöstutkimus koostuu neljästä osatyöstä. Osatyö **I** käsittelee tutkimusjakson aikana tapahtunutta Pirkanmaan vesiepidemiaa. Marras-joulukuun vaihteessa 2007 Pirkanmaalla noin 12 000 asukkaan juomavesi saastui jätevedellä. Tästä aiheutui harvinaisen raju gastroenteriittiepidemia, jossa nähtiin vaikeita ja usean patogeenin yhdessä aiheuttamia tauteja. Kaikkiaan epidemiaan liittyen hoidettiin Taysissa 115 lasta, joista 65 saatiin tutkimukseen. Valtaosalla lapsista todettiin vaikea-asteinen gastroenteriitti, ja ulosteesta löytyi samanaikaisesti useita suolistopatogeenejä.

Voitiin todeta, että hyvin voimakas altistuminen enteropatogeenisille viruksille ja bakteereille aiheuttaa samantyyppisiä vaikeita oireita mikrobista riippumatta.

Osatyöt **II**, **III** ja **IV** ovat eteenpäin suuntautuvia tutkimuksia. Osatyöt **II** ja **III** käsittelevät lasten rotavirus- ja norovirustautien epidemiologiaa kahden vuoden seurantajakson aikana. Ensimmäisenä kautena (2006–2007) rotavirusten aiheuttamien tautien määrä sairaalatasolla oli poikkeuksellisen pieni (38%), ja norovirusten osuus (34%) oli korostunut. Ensimmäisenä "heikkona" rotaviruskautena nähtiin tavallisimman G1P[8]–genotyypin lisäksi huomattavan paljon genotyyppiä G9P[8]. Toinen seurantavuosi (2007–2008) oli tavanomaisempi rotavirusten esiintyvyyden ja genotyyppien suhteen: 62% lastenklinikalla nähdyistä gastoenteriiteistä oli rotaviruksen aiheuttamia, ja tavallisin genotyyppi oli G1P[8]. Molempien seurantavuosien aikana havaittiin selkeät rotavirusgastroenteriittikaudet alkuineen ja loppuineen.

Seuranta-aikana rotavirusrokotteet olivat juuri tulleet saataville omalla kustannuksella hankittaviksi. Ensimmäisenä markkinoille tuli ihmisperäinen genotyyppiä G1P[8] sisältävä rokote (Rotarix®), ja pian sen jälkeen rokote, joka sisältää vasikan rotaviruksen kanssa reassortoidut G-tyypit 1-4 ja P-tyypin [8] (Rotateq®). Ensin mainitun rokotteen kattavuus nousi seuranta-aikana 29%:iin, ja toisen 6%:iin; yhteensä rokotuskattavuus lisääntyi seuranta-aikana 35%:iin kohteena olevasta ikäluokasta. Tämänkin tasoinen rokotuskattavuus mahdollisesti vähensi rotavirusgastroenteriittien esiintymistä sairaalatasolla rokotteen suoran tehon ja viruksen tartuntareittien vähenemisen myötä.

Molempina seurantakausina tavallisin norovirusgenotyyppi oli GII.4, joka onkin vallinnut koko maailmassa 2000-luvulla. Norovirusgastroenteriitit esiintyivät pääasiassa talvella, mutta kausittaisuus poikkesi rotavirusten esiintymisestä. Kun tautien vaikeutta tarkasteltiin 20 pisteen asteikolla, jossa $\geq$11/20 pistettä luokitellaan vakavaksi gastroenteriitiksi, todettiin että rotavirusgastroenteriiteistä 31% ja norovirusgastroenteriiteistä 18% oli vakavia.

Laskelmat rotavirus- ja norovirusgastroenteriittien aiheuttamista kustannuksista vanhemmille ja yhteiskunnalle esitellään yhteenveto-osassa. Kun tutkimuksemme tulokset laajennettiin koko Suomen mittakaavaan, todettiin norovirusten aiheuttaneen vuoden 2007 hintatason mukaan laskettuna sairaalatasolla noin 1 801 000€ ja rotavirusten noin 3 714 460€ vuosittaiset kustannukset.

Osatyössä **IV** kuvataan vuosina 2009–2011 nähty nopea lasku rotavirusgastroenteriitten määrässä lapsilla sen jälkeen, kun rotavirusrokote tuli mukaan Suomen kansalliseen rokotusohjelmaan.

Väitöskirjatyössä selvitettiin rotavirusten epidemiologista tilannetta Suomessa lapsilla juuri ennen kattavien rotavirusrokotusten aloittamista. Tutkimuksella saatiin uutta tietoa norovirusten merkityksestä lasten kausittaisen ripuli-oksennustaudin aiheuttajana. Tuloksia on käytetty pohjana vertailtaessa rotavirusrokotteen tehoa ja merkitystä Suomessa. Kuten oletettavissa olikin, sen jälkeen kun rotavirusgastroenteriitit pienillä lapsilla on rokotuksilla saatu lähes häviämään, nousivat norovirukset merkittävimmiksi ripuli-oksennustaudin aiheuttajiksi. Norovirusgastroenteriitit ovat niin yleisiä ja voivat olla niin vakavia, että imeväisikäisten ja pienten lasten rokottaminen norovirusta vastaan on perusteltua, kunhan rokote tulee saataville. Tällaista rokotetta kehitetään parhaillaan.

List of original publications

The thesis is based on the following publications, which are referred to in the text by Roman numerals as listed below. The original articles are reprinted with the permission of the copyright holders.

I   Räsänen S, Lappalainen S, Kaikkonen S, Hämäläinen M, Salminen M, Vesikari T. Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak. Epidemiol Infect. 2010 Sep;138(9):1227-34.

II Räsänen S, Lappalainen S, Halkosalo A, Salminen M, Vesikari T. Rotavirus gastroenteritis in Finnish children in 2006-2008, at the introduction of rotavirus vaccination. Scand J Infect Dis. 2011 Jan;43(1):58-63.

III Räsänen S, Lappalainen S, Salminen M, Huhti L, Vesikari T. Noroviruses in children seen in a hospital for acute gastroenteritis in Finland. Eur J Pediatr. 2011 Nov;170(11):1413-8.

IV Hemming M, Räsänen S, Huhti L, Paloniemi M, Salminen M, Vesikari T. Major reduction of rotavirus, but not norovirus, gastroenteritis in children seen in hospital after the introduction of Rotateq vaccine into the National Immunization Programme in Finland. Eur J Pediatr. 2013 Jun; 172(6):739-46

Additional data on the burden of disease and associated costs of norovirus gastroenteritis in children in comparison with rotavirus are presented in the Results section below.

Abbreviations

AdV	Adenovirus
AGE	Acute gastroenteritis
AiV	Aichi virus
CRP	C-reactive protein
CSS	Clark severity score
ELISA	Enzyme immunoassay
EM	Electron microscopy
HBGA	Histo-blood group antigen
HBoV	Human bocavirus
HCoV	Human coronavirus
HuCV	Human calicivirus
NIP	National immunization program
NoV	Norovirus
ORS	Oral rehydration solution
RT-PCR	Reverse-trascription polymerase chain reaction
RV	Rotavirus
SaV	Sapovirus
SR	Sedimentation rate
SRV	Small round virus
THL	National Institute for Health and Welfare
UNK	Unknown
VLP	Virus-like particle
VSS	Vesikari severity score

Contents

1   Introduction

Acute gastroenteritis (AGE) is one of the leading causes of mortality in children worldwide and an important cause of morbidity and hospitalization in developed countries (1-4). In developed countries, viruses cause the majority of AGE cases. In high-income countries, mortality from AGE is low because rehydration therapy and hospital facilities are available (2,3,5).

The etiology of AGE was largely unknown until the discovery of rotaviruses. Cases caused by enteropathogenic bacteria were partially recognized, but the other causative agents could not be identified. "Cholera infantum," or enteritis of young children, was a common cause of death in Europe, and even as late as the 1940s in United Kingdom 40 of 70 admissions per week for enteritis in children less than 15 months of age led to death (6). Diarrheal diseases were seen particularly in hot summer months ("summer diarrhea"), but a syndrome called "winter vomiting disease" was described in 1929 by Zahorsky (7). It was suggested to be a viral infection. As bacterial gastroenteritis diminished along with improved hygiene, sanitation, and food and water handling, but non-bacterial gastroenteritis remained, viruses stood out as the most important causes of AGE in children (8-10).

Rotavirus (RV) was discovered in 1973 by electron microscopy (EM) of intestinal biopsies and from stools soon after (6,11-13). Progress was relatively slow because EM was not readily available for clinical studies. The importance of RV as a causative agent of AGE in small children was established in the following years. In Finland, RV was found to cause 54% of AGE in hospitalized young children, and adenovirus (AdV) was identified as causing about 5% (8-10). An etiological study of AGE in children was conducted at Tampere University Hospital in 1977-1978 (10). For this study, up-to-date diagnostic methods were established for the diagnosis of both bacterial and viral causes of AGE. For RV, this meant initially EM, but this method was later replaced by radioimmunoassay and enzyme immunoassay (ELISA) to detect RV antigen in stools. With these methods, it was possible to find RV in 54% of the hospitalized AGE cases. Bacteria were found to play a role in less than 10% of the cases. About one-third of the AGE cases remained unclear as regards etiology.

Norwalk virus was actually discovered before RV in examination by EM and immunoelectron microscopy (IEM) of stools from an outbreak of AGE in a school in Norwalk (14). Norwalk also gave the name to norovirus (NoV), as it is called today. Occasionally small round structured viruses (SRVs), probably NoVs, were seen by EM in the stools of children with AGE, but the sensitivity of EM was not sufficient to detect these viruses in most cases. NoVs cannot yet be cultured today, and the antigen detection tests are insensitive; the only reliable diagnostic method is RT-PCR. Since RT-PCR has become available, the importance of NoVs as a frequent cause of AGE in outbreaks was understood, and the importance of NoVs as a causative agent of seasonal AGE in children has gradually become acknowledged.

Based on a prospective material of AGE from birth to 2½ years of age collected by Tarja Ruuska (15), it was found – in collaboration with the National Institute of Health, who owned an early serological test – that 50% of Finnish children had become seropositive for NoV by 2-2½ years of age (16). However, the NoV detected serologically could not be linked to AGE because there was no sufficiently sensitive detection method for NoVs. RT-PCR using multiple primers for NoVs became available in the late 1990s and was used on a collection of stools from a rhesus-human reassortant tetravalent RV vaccine trial in Finland in 1993-1995. It was found that NoVs (then called Norwalk-like viruses or NLVs) were responsible for 20% of AGE in children under the age of two, and were actually just as common as RVs (17). This study was the first one to show the true importance of NoVs as causative agents of endemic AGE in young children.

The cornerstone of treatment of AGE, regardless of etiology, is rehydration and correction of the electrolyte balance, which often requires hospitalization, especially in small children. Gastroenteritis viruses are highly contagious, and their spread is difficult to prevent through hygiene only. Therefore, vaccination against AGE is a logical means of prevention.

At the time when we launched this study, two vaccines against RV had just become commercially available in Finland. Vaccine coverage reached 35% of the target population at the end of our follow-up period in 2008. Later, in 2009, a vaccine against RV was introduced in the national immunization programme in Finland, and RV vaccine coverage rose rapidly reaching 91% in 2012. The extension study on RV epidemiology in 2009–2011 shows the effect of the vaccination as a decrease in RV cases.

2 Review of the literature

2.1 Acute gastroenteritis in children

Acute gastroenteritis (AGE) is one of the infectious disease entities that cause major morbidity and mortality in the world: after pneumonia, diarrheal diseases are the second most important infectious cause of death in children under five years of age (1,18). It is estimated that AGE causes 580 000 – 750 000 deaths in children of this age in the world every year (19). Most of these fatal cases occur in countries with poor health care systems and a lack of safe water and sanitation, e.g. in Sub-Saharan Africa and Southeast Asia. In developed countries, deaths due to AGE are very rare. However, AGE viruses circulate in both developed and poor countries, causing an estimated 1.5 billion AGE cases every year in children and adults (20). As a consequence, the disease burden in morbidity, heath care utilization and expenses caused by AGE is enormous.

In health care, AGE is one of the most common diagnoses when treating acutely ill children. Until lately, AGE was also one of the most common reasons for hospitalization in children, but in the countries where the rotavirus (RV) vaccines have been adopted in universal use, the scale of pediatric infectious diseases in inpatients has changed. These vaccines have also had an impact on childhood deaths (21). If the remaining common AGE causative agents could also be prevented, a vast amount of human suffering and money could be saved.

2.1.1 Causative agents

In children, AGE is most often caused by viruses (22-24). Especially in Western countries, bacterial AGEs are usually associated with travelling or exposure to contaminated food or water. Seasonal bacterial AGE, or "summer diarrhea", by which name it was also known, has become more or less disappeared nowadays (25).

Among AGE viruses, RV was the most important AGE-causing agent in children before introduction of vaccines against RV (26). RV AGE is mainly a disease of small children less than three years of age, and in them it is often severe enough to require hospital treatment. Today, in countries where RV vaccines are widely used, the incidence of RV AGE has diminished in the child population, and noroviruses (NoVs) may be considered to be the most important causative viruses of AGE to date in the world (27). In addition, sapoviruses (SaV), adenoviruses (AdV) and astroviruses are among the regularly reported viral causative agents of AGE (28-31) (see below in Chapter 2.4). SaVs cause epidemics, especially in small children and the elderly, and most children have probably been infected by the age of five. The epidemiology of SaVs is not well known, perhaps because they are not usually detected in clinical practice. Other, non-viral causes of AGE still are common and important, especially in developing countries (1,32).

2.1.2 Clinical features

AGE is a clinical diagnosis based on an acute onset of symptoms that include loose stools or diarrhea, vomiting, nausea and stomachache, with or without fever. The defined criteria of AGE often used in studies include at least three loose stools within a 24-hour period or forceful vomiting.

As a whole, RVs cause more severe symptoms than other AGE causative agents (28,33). Dehydration occurs, especially in small children or the elderly, and the risk of substantial dehydration increases along with the duration of the AGE symptoms. However, in infants, dehydration may develop rapidly, especially if robust vomiting and diarrhea occur simultaneously.

Bloody diarrhea is not typical for viral AGE, but more suggestive of bacterial disease (8). If laboratory markers of infection are detected – they usually are not – the CRP and SR are usually normal or slightly elevated in viral AGE, but in bacterial AGE they may be substantially elevated, depending on how invasive the disease is.

Treatment of AGE does not usually require detection of the causative agent, because the treatment is the same independent of the causative virus. The causative agent is studied mainly in hospitals due to the severity of an individual AGE case, to support epidemic control in the ward, or for local epidemic surveillance.

2.1.3   Assessing the severity

The severity of AGE is assessed mostly for study purposes, and usually not for clinical practice. Scales for assessing severity were introduced for RV vaccine studies.

The most widely used severity scale is a 20-point scale also known as the Vesikari severity scale (VSS), introduced by Ruuska and Vesikari in the 1980s (34). In this scale, the intensity and duration of diarrhea and vomiting, the degree of fever and dehydration, and the treatment needed are considered and rated (Table 1). AGE is considered severe if it scores ≥11 points and moderately severe if the severity score is 7-10 points.

Another AGE severity scale used is the Clark severity scale (CSS), which is a 24-point scale. This scale measures the intensity and duration of diarrhea and vomiting, degree and duration of fever, and altered behavior and its duration, and the AGE is categorized as moderately severe if it scores 9-16 points, and severe at >16 points (35,36). Both severity scales are presented in Table 1.

Using the VSS with a cutoff of 11/20, more AGE cases can be classified as severe than by using the CSS with a cutoff of 16/24 (37). In evaluating the efficacy of a vaccine, it probably is reasonable to use a scale that overestimates the severity of AGE episodes rather than one that may underestimate it. For RV vaccination, the endpoint is protection against severe AGE.

Symptom or sign		Points		
		1	**2**	**3**
Duration of diarrhea (days)	VSS	1-4	5	≥ 6
	CSS	1-4	5-7	> 7
Max. no. of diarrheal stools / day	VSS	1-3	4-5	≥ 6
	CSS	2-4	5-7	> 7
Duration of vomiting (days)	VSS	1	2	≥ 3
	CSS	2	3-5	> 5
Max. no. of vomiting episodes /day	VSS	1	2-4	≥ 5
	CSS	1-3	4-6	> 6
Max. degrees of fever	VSS	37.1 - 38.4	38.5 - 38.9	≥ 39
	CSS	38.1 - 38.2	38.3 - 38.7	≥ 38.8
Duration of fever (days)	VSS	-	-	-
	CSS	1-2	3-4	≥ 5
Dehydration	VSS	-	1-5 %	$\geq 6\%$
	CSS	-	-	-
Treatment	VSS	Rehydration	Hospitalization	-
	CSS	-	-	-
Behavioral symptoms: description	VSS	-	-	-
	CSS	Irritable/ less playful	Lethargic/ listless	Seizure
Behavioral symptoms: duration (days)	VSS	-	-	-
	CSS	1-2	3-4	≥ 5
Max. score	**VSS**	**20**		
	CSS	**24**		

Table 1. The 20-point severity scale (Vesikari severity scale, VSS) and the 24-point severity scale (Clark severity scale, CSS).

2.1.4   Clinical assessment and treatment

The severity of dehydration of a child is assessed by clinical signs: general condition, fatigue, alertness, turgor of skin, dryness of lips and mouth, and in infants the tonus of the fontanel. Anamnestic information about frequency of vomiting and diarrheal stools, behavior and fatigue at home, amounts of drinking and urinating, and duration of the symptoms are also important. The younger the

child is, the greater the risk of severe dehydration and the faster it develops. A dehydration rate of <5% is usually considered mild or moderate, 5-10% severe, and >10% critical. Laboratory tests for evaluating the electrolyte balance and acidosis can be helpful, but they usually are not essential in making treatment decisions.

In treating AGE, it is essential to restore and maintain the balance in liquids and salts: the loss from vomiting and stools must be replaced perorally or via nasogastric tube, or failing that, intravenously. Peroral rehydration is the treatment of choice whenever possible, because it is safer than intravenous rehydration, as well as more practical in the treatment of both outpatients and admitted patients. In addition, peroral rehydration can be administered also outside hospitals. Peroral rehydration therapy by oral rehydration solution (ORS) for dehydrated children at the hospital level was introduced at the Tampere University Hospital as the first hospital in Finland and one of the first in Europe, after the work of local researchers in improving the solution and studying its effectiveness and benefits in clinical use (38-40). Today, there are several commercial products available for oral rehydration. The composition of fluid in these products is designed to be as ideal as possible for replenishment of salts, glucose and water. Some of the commercial ORS products also contain probiotic bacteria, which have been shown to be advantageous in shortening the course of the symptoms of AGE (41-43) and are generally recommended in treating AGE in children (44,45).

The degree of dehydration is not an essential criterion in choosing the route of rehydration. Rehydration solutions can be given by mouth or nasogastric tube. Intravenous dehydration may be needed, for example because of excessive vomiting. If the rehydration can be arranged in an outpatient clinic, it usually takes two to six hours to see whether the child gets better, and, if so, administration of liquids can be continued at home. Sometimes hospitalization is needed, mainly if the child is too ill to take liquids without vomiting instantly, or if the general condition does not seem to improve during outpatient clinic treatment.

There are no therapeutic drugs for viral AGE in clinical practice, but the treatment consists of rehydration and maintenance of nutrition. Drugs affecting gastrointestinal motility are generally not used in children. Zinc supplementation, which may shorten the length of AGE episode, is used especially in developing countries (46-48). Antiviral agents against AGE have been studied in vitro and used in clinical studies. A broad-spectrum anti-infective agent nitazoxanide has been most promising candidate (49,50) and can be considered in the treatment of immunocompromised AGE patients.

2.2   Rotavirus gastroenteritis in children

2.2.1   Rotavirus

2.2.1.1   Structure

The RVs are non-enveloped double-stranded RNA viruses belonging to the *Reoviridae* family. The genome contains 11 gene segments that encode the structural proteins VP1-8 and the non-structural proteins NSP1-6 (51,52).

The RV capsid is formed by three protein layers (Figure 1). The outer capsid of the RV virion consists of two proteins, VP7 and VP4, both of which are immunogenic antigens and induce type-specific neutralizing antibodies. The VP7 proteins form the shell of the RV virion, and VP4 proteins spike outwards from the capsid and act in the attachment of the virion. Trypsin cleavage of VP4 into VP5 and VP8 enhances viral penetration to the target cell and infectivity of the virus (51,53). The inner capsid of the RV virion is formed by VP6, which is a highly conserved and abundant protein inducing heterotypic cross-protective immunity in humans (54). The innermost layer of the virion core consists of the proteins VP1, VP2 and VP3.
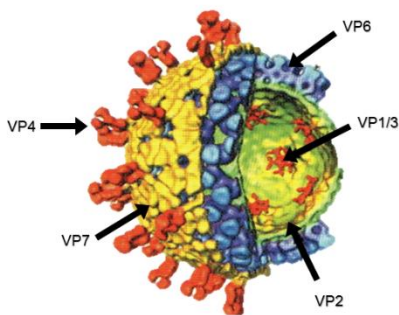
Figure 1.   The protein structure of rotavirus capsid. Modified from (52).

2.2.1.2 Classification: groups and genotypes

Human enteropathogenic RVs are divided into groups (or serogroups) A, B and C based on the viral protein VP6 (51) (Figure 1). Group A RVs are the common causative agents of AGE, responsible for more than 90% of all the RV AGE in humans, while the others are of minor significance in the epidemiology of RV (51,55).

Group A RVs are divided into genotypes (or serotypes if classified by antigens) by their outer capsid structural proteins VP4 and VP7 (51). The genotype of RV is indicated as G- and P-types. The G-type is defined by the structural protein VP7 and the P-type by VP4 (Figure 1). The G- and P-types are indicated with numbers, of which the latter, the P genotype, is given in brackets: for example G1P[8] (51,56). At present, 27 G-types and 35 P-types have been described (57). The VP7 genotypes G1, G3, G4 and G9 most often present with the VP4 genotype P[8], and G2 usually presents with P[4] (51,55).

2.2.2 Epidemiology

Since RV was discovered from the duodenal mucosa of children with AGE in 1973 (11), it was soon recognized to be the most important causative agent of AGE in children, at least in developed countries (6,10,58). Practically every child is infected by the age of five years: in serological studies at least 95% of children became seropositive for RV by the age of five (59,60). However, only 20-30% of children suffer from RV AGE that requires health care contact (61).

RV AGE is seen equally distributed all over the world, independently of the level of sanitation or population density. However, the consequences of AGE are very different in developed and developing countries. RV AGE is one of the leading causes of child mortality in the world. Before the RV vaccination, it was estimated that every year about 450 000-610 000 children under five years of age died because of RV AGE, accounting for 5% of total childhood deaths in that age group (3,58,62,63). Most of the deaths occur in sub-Saharan Africa and India (3,62,64), whereas fatal AGE cases are very rare in Western countries. In the countries, in which universal RV vaccination has not been implemented, RV continues to be a leading cause of severe AGE and childhood hospitalization (1,18,31,32).

Peak incidence of RV AGE is between the ages of 6 and 23 months (10,61,65-67): 58-77% of all RV AGE cases occur in children of that age group (65,68). Most RV hospitalizations also occur in the same age group (69,70).

2.2.2.1 Seasonality

In temperate climates, RV AGE seasons typically occur during the winter months, beginning in late autumn or winter and lasting until the spring (51,71-73). This seasonal pattern is seen in both the northern and southern hemispheres, most clearly in Europe, North America and Oceania, the RV seasonal peak occurring in the winter months of each. In tropical countries, the seasonal pattern is not seen or is not as definable. On the other hand, there are countries of temperate climate with a year-round occurrence of RV AGE and tropical countries with RV seasonality (51,71,72).

The reason for seasonality is not completely understood: there is probably no single explaining factor, but the seasonality is the result of multiple co-predictors including the temperature, humidity and yearly rainfall, altitude, population density and behavioral factors, and perhaps the economic income of the country (72). The economic status of countries does not explain the seasonal or year-round occurrence of AGE, because in countries with similar income levels, seasonality exists or is lacking, depending on the country's distance to the equator (72).

2.2.2.2 Predominating genotypes

The five major circulating RV genotypes in Europe, North America and Australia, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], are responsible for more than 90% of the RV AGE cases in these areas (74-82). The predominant genotype in these parts of the world in the long-term perspective is G1P[8] (77,79,80). However, the epidemiologic situation is not constant: natural variation occurs. Especially genotype G9P[8] RVs have taken local predominance for single seasons in the 2000s (74,75,77-79,81,83-85). Such temporary predominance can also be seen with other major genotypes (77,79,86,87). Even though the variation in and scale of the circulating RV genotypes is greater in Africa and Asia, G1P[8] and G9P[8] are usually found to be the dominant RV types there as well (31,85,88-91). The sixth relatively common RV genotype globally is G12P[8], which emerged in the 1990s

and 2000s (82,92). Of the other genotypes, G5P[8] in South America and G8P[6] in Africa, for example, are frequently seen in circulation (93-95).

In REVEAL study the presenting RV genotypes in seven European countries (Belgium, France, Germany, Italy, Spain, Sweden and United Kingdom) were studied during the same AGE season. In all these countries, the five main genotypes accounted for 98% of the RV AGE cases, but the predominant RV genotype varied from country to country even within Europe: in Belgium, Spain, United Kingdom and Sweden it was G1, in France and Italy G9, and in Germany G4 (77). It seems that the global epidemiology of RVs is different from, for example, that of influenza viruses: even if the seasonal pattern in different countries is alike and temporally connected, the epidemics are independent from each other.

RV vaccines have changed the molecular epidemiology of RV in the countries in which they have been adopted, as discussed below in Chapter 2.5.2.

2.2.3   Clinical picture of rotavirus gastroenteritis

The symptoms of RV AGE consist of watery diarrhea, vomiting and fever. Because of the wide spectrum of symptom severity, the causative agent of an individual AGE episode cannot be reliably differentiated by its symptoms (96). In the bigger picture, RV AGE is often more severe compared to AGE caused by other viruses: the risk of dehydration is higher, fever is seen more often, and RV AGE more often leads to hospitalization (8,12,66,70,97). Clinically, RV AGE caused by different genotypes cannot be distinguished, although it has been proposed that G1P[8] and G9P[8] cause more severe symptoms on average than other RV genotypes (98).

Diarrhea with or without vomiting is the predominant feature in RV AGE. In a Finnish community-based prospective study of AGE in children less than two years of age, Pang et al showed that there was a difference between AGEs caused by different viruses: diarrhea was more pronounced in RV AGE, whereas vomiting was seen more often in NoV AGE (28).

The incubation period of RV AGE is typically one to three days, with an average of two days (51,99,100). The symptoms begin acutely, most often with vomiting followed by watery diarrhea. Sometimes stomachache precedes the major symptoms. Vomiting lasts on average for two to three days, and diarrhea for four to five (55,100), but there is a great deal of variation. Fever is often seen, and

febrile seizures are seen in children with a predisposition for them. Bloody diarrhea is rare (8,55,100). Antigenemia and viremia can be seen, also in immunocompetent children, but systemic complications are rare (55,101-104).

Many children, however, are infected by RV without marked clinical symptoms. Especially infants less than six months of age seem to have protection by maternal antibodies, and if infected during these first months, the infection often remains silent (51,59,105). Also in older children RV infection can sometimes be passed with nonspecific symptoms or no clinical symptoms at all (55,100), usually if the child has already had a primary RV infection (51,106,107).

2.2.4 Transmission and shedding

The main route of transmission of RV AGE is fecal-oral (51,55), but it can also occur via respiratory droplets containing mucus and viruses (51,55,97). The spreading of the infection is very difficult to control. The virus is shed in large numbers, up to 10^{11} infectious viral particles/mL in stools of an infected person and, on the other hand, the contagious dose of RV is very small: about 10 viral particles (55,108). Besides these, knowing that RVs are highly resistant to environmental factors such as temperature and pH (100), it is easy to understand why RV AGE is very easily transmitted, especially at day-care centers, where young children prone to infection are in close contact with each other, or in a hospital ward treating children with and without AGE.

Shedding of viruses in stools have been demonstrated for about two or three weeks (109) by RT-PCR (55,110,111), but in immunocompromised persons the shedding can last as long as months (109,110).

2.2.5 Diagnosis of rotavirus gastroenteritis

The presence of RV is commonly detected in stools by the ELISA antigen test or RT-PCR. ELISA tests are commercially available and fast to perform. The sensitivity of ELISA tests is good (112), but false positives can also occur. Compared to the ELISA test, RT-PCR finds more RV-positive stool specimens, but the additional cases are mild ones; the sensitivity of the two methods is about the same in finding severe cases (112). This suggests that the sensitivity of ELISA is good enough to be used at the hospital level and in vaccine efficacy studies, and the studies conducted using ELISA are comparable to the ones using RT-PCR.

However, the ELISA test cannot be used if the size of the stool sample is very small or if the stool is soaked into a diaper. Furthermore, ELISA cannot determine the RV genotype. Compared to ELISA, RT-PCR is better in detecting especially RVs of genotype G2 and G4 (112), and perhaps of more uncommon strains as well.

The RT-PCR method is highly sensitive (113), and the test can be done from a minimal amount of stool, such as from diapers or a rectal swab. The genotype of RV can be determined by sequencing the VP7 and VP4 coding regions (113). Until lately, RT-PCR testing has mainly been used in research, but along with rapid-PCR techniques, the test is becoming more commonly available, also in clinical practice.

2.2.6   Immunity

Protective immunity responses to RV challenge are complex and not yet fully understood. Both antibody- and cell-mediated immunity play a role in this (51,107,114). Of the antibodies, serum RV-specific IgA against VP6 is probably the single most important mechanism for protective immunity against subsequent disease, but not infection (107). The level of serum-neutralizing IgG antibodies against VP7 and VP4 also correlates with protection (51,59,114,115). Antigenemia and viremia often occur during acute RV infection (101,102,104), enhancing and inducing antibody production and memory B-cell production.

The first natural RV infection provides substantial protection against severe re-infections, and a second infection results in practically complete immunity against moderate to severe infections (59,97,105,106,116). Both symptomatic and asymptomatic infections induce immunity: also neonatal RV AGE provides fairly good protection against subsequent symptomatic RV AGE (105). Serotype-specific immunity results in especially efficient protection against subsequent infections caused by a similar RV strain, and efficient cross-protection occurs especially after the second infection (59,97,115). With ageing or weakening of the immune system for other reasons, protective immunity against RV also commonly vanishes.

2.2.7   Significance

2.2.7.1   Morbidity and mortality

In Europe, before the introduction of RV vaccines, the estimated annual rate of RV AGE was 2.07-4.96 per 100 children (the incidence rate of all causes of AGE being 4.22-16.82 per 100 children), RV causing up to two-thirds of all AGE hospitalizations and emergency clinic treatments and one-third of primary care consultations for AGE (65). It was estimated that RV annually caused 3.6 million AGE episodes in European Union countries, of which about 700 000 children annually needed medical care and about 90 000 children (or about 1 of 54 children) were hospitalized because of RV AGE occurring before the age of five (5). The situation remains the same in the countries where vaccines against RV have not been adopted.

In Finland, the epidemiology of RV was first studied in 1977-1978 (9) and subsequently in large RV vaccine studies (28,117). It was estimated in the 1990s that RVs cause about 2000 hospitalizations annually of children less than five years of age in Finland; in other words, 3% of Finnish children were admitted to hospital because of RV AGE (61). In the study by the National Institute for Health and Welfare (THL) before introducing RV vaccination in Finland, it was estimated that RV caused an annual 2400 hospitalizations, 3700 outpatient clinic treatments and 9000 consultations in primary health care in children less than five years of age in Finland (118).

It was estimated that globally RVs caused about 453 000 deaths every year in children less than five years of age before introduction of RV vaccines (3). In high-income countries, such as Finland, deaths for RV AGE are very rare, at the level of one in 50 000 cases (58). The mortality and morbidity caused by RV has diminished in the countries with high coverage of the protective vaccines (21,119-121) (see below in section 2.5.2).

In pediatric hospitals, RV can readily cause nosocomial epidemics that are very difficult to abort. In an European review before universal vaccinations it was estimated that RVs annually cause 7.0-15.8 nosocomial infections per 1000 hospital days in children younger than two and 0.7-8.1 nosocomial infections per 1000 hospital days in children younger than five (122). Besides AGE in children, RVs sometimes cause also AGE outbreaks (66,123,124) and sporadic epidemics in institutional settings. The waning of acquired immunity against RV predisposes

elderly persons to infection, and if the virus escapes to prone settings, controlling the outbreak before all the prone persons have caught the infection is not simple.

RV vaccination was introduced in the Finnish national immunization program (NIP) in 2009 and, since then, vaccine coverage has been more than 90% (125). In the following years, RV AGE cases in small children diminished rapidly, and hospitalizations caused by RV AGE practically disappeared in the children who had received the vaccination (see Chapter 2.5.2).

2.2.7.2 Economic significance

The RV AGE episodes are costly both for society and for individual families. For society, the major expenses are the cost of hospitalizations and lost working days of the parents. These are also the costs which can be estimated. The expenses from visits in primary health care and private clinics are much more difficult to estimate because there are no commonly used recording systems available for such an evaluation. Besides the societal costs, AGE episodes result in direct, out-of-pocket costs for the families, for example for the necessary medication, transportation, and extra laundry and diapers.

The costs of RV AGE in children less than five years of age in seven European countries (France, Italy, Spain, Sweden, Belgium, Germany and the United Kingdom) were studied in the REVEAL study in 2004-2005. The societal costs for RV AGE hospitalizations varied from €1525 (France) to €2101 (Sweden) per episode; the costs for payers varied from €1221 (United Kingdom) to €1830 (Sweden). The societal costs for RV AGE treated as outpatients in an emergency department ranged from €334 (France) to €770 (United Kingdom) per episode, and those for families ranged from €80 (France) to €476 (United Kingdom). There were no cases from Belgium and Germany in this category. The societal costs for RV AGE treated in primary care varied from €473 (Belgium) to €166 (Spain) per episode, and those for families varied from €17 (Spain) to €110 (Germany) (126).

The mean number of days lost from work because of a child's AGE varied from 2.3 (France) to 6.4 days (Germany) with hospitalized children, from 2.5 (France) to 4.4 days (Spain) with children treated in emergency care, and from 3.4 (France) to 7.5 days (United Kingdom) with children treated in primary health care in the same REVEAL study material (126,127).

Altogether, the costs of AGE are not easy to measure or to compare between different countries because of different treatment practices, different social benefits, and different maternal/paternal leave and sick leave systems.

2.3　Norovirus gastroenteritis

2.3.1　Norovirus

2.3.1.1　Structure

NoVs are small, nonenveloped, round viruses with a linear, positive sense, single-stranded RNA encoding three open reading frames (ORFs). Of these, ORF1 encodes non-structural proteins, including the viral polymerase, and ORF2 and ORF3 encode the major and minor structural capsid proteins VP1 and VP2 (128,129). The VP1 consists of the N-terminal region, the shell (S) region, and protruding (P) regions. The virus capsid is formed by 90 VP1 dimers, which are arranged as shown in Figure 2.

NoVs do not grow in cell cultures in vitro, and NoV particles cannot be produced by standard methods. However, when expressed in baculoviruses and produced in insect cells, the NoV capsid protein spontaneously forms a virus-like particle (VLP) that is stable and antigenic and can be used as an antigen in serological assays and vaccine research (130-132).

2.3.1.2　Classification: Genogroups, genotypes and variants

NoVs belong to human caliciviruses (HuCVs). Of the family *Caliciviridae*, the genus *Norovirus* can be divided into at least five genogroups (134), of which genogroups 1 (GI) and 2 (GII) are the common human pathogens. Also genogroup 4 (GIV) viruses are able to infect humans, but they are rare as AGE causative agents (133,135).

The NoV genogroups are further divided into genotypes indicated by numbers, e.g. GI.1 (134). More than 31 genotypes of noroviruses are known, and of these, particularly genotype GII.4 seems to be under constant genetic variation with new variants (or strains) emerging every two or three years in the 2000s (134). The mechanisms NoVs use to increase the variation are mutation and recombination.
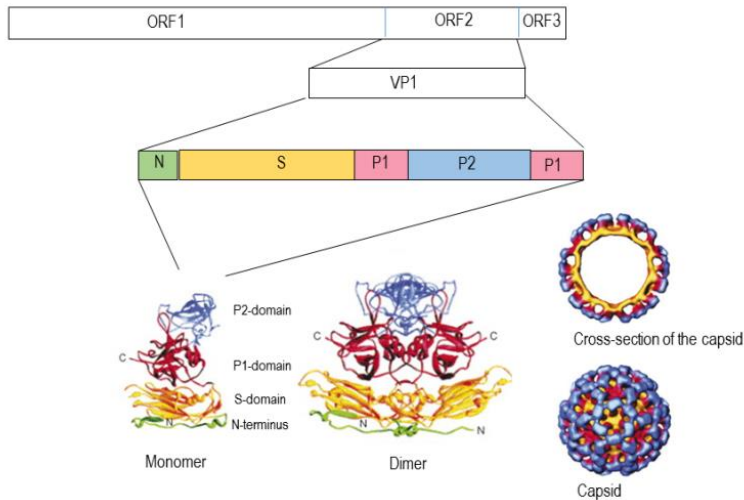
2.3.2 Epidemiology

For decades, probably the majority of cases of what was formerly the clinical syndrome "winter vomiting disease" – sharply beginning and short lasting illness with vomiting and diarrhea – have actually been NoV AGE (130,135). In children, the clinical features of NoV AGE are often not so typical, and it took a long time before it was realized that NoV also causes seasonal AGE in children. The seasonal NoV AGE in children was mainly recognized in the 2000s, first shown in Finnish rotavirus vaccine studies (17,28). Nowadays, NoVs are generally recognized as the major causative agents of viral gastroenteritis in the world (27,136-139). They affect people of all ages and everywhere in the world. However, it is not easy to evaluate the exact real incidence or total burden of NoV AGE because laboratory studies are not commonly done, except in outbreaks.

2.3.2.1 Seasonality

In moderate-climate countries, the NoV season traditionally begins in winter and lasts until spring, between October and March in the northern hemisphere (140,141). About three-quarters of NoV cases occur in these cool months.

The explanation for the seasonality of NoV AGE is not known, but such factors as rainfall, cool temperatures and the emergence of new variants have been proposed (140,142,143). It seems reasonable to think that there is also an association between AGE seasons and people's behavior: holiday seasons versus school and day care seasons with crowding indoors and public vehicles.

2.3.2.2 Outbreaks

NoV is the most important causative agent of AGE outbreaks (27,139,144). In foodborne outbreaks, the contamination can originate in the source of the food, like contaminated seafood (originating from contaminated water), frozen berries (irrigated with contaminated water) or contamination of the raw-consumed products via handlers' hands, or it may have happened in the food-serving facilities, in which case the source of contamination is commonly the food handlers (145-150). Waterborne outbreaks can occur from contaminated drinking water (151-154) or recreational water (155-158). However, finding the source of the outbreak is often difficult. Even if the laboratory methods for detecting the viruses are sensitive enough for human stool samples, in which the viral load is high, the sensitivity is often not high enough to detect NoVs in food, water or the environment: only a few virus particles are needed to cause transmission of the infection, but much larger doses of viruses are required to exceed the detection limit of laboratory tests. The secondary infections from person to person can confuse an investigation into the origin of the outbreak (159).

NoVs commonly cause epidemics also in institutional settings such as child care centers, elderly homes, and hospitals (159-161). These outbreaks are especially difficult to abort because of the extremely efficient transmission of NoVs and their longevity on surfaces (139,146,159,162). Furthermore, the vulnerability of elderly people makes the AGE more severe and potentially fatal. The small children, on the other hand, are naïve in immunity, and transmission occurs easily via diapers and hands. Elderly people and children may also be unable to practice good hand hygiene, and contaminated hands or objects might be easily taken to mouth.

Furthermore, in these groups and other immunocompromised persons, the shedding of the virus in stools is prolonged (163-166).

2.3.2.3 Predominating genotypes

In seasonal NoV AGE and in AGE outbreaks, GII.4 has been the predominating genotype since the 1990s (141,167-169). This genotype is constantly under active evolution, and, as a consequence, new variants of NoV GII.4 have appeared and spread, causing epidemics throughout the world every few years (134,141,170-173). Also other genotypes of NoVs are seen in seasonal AGE, the majority of them also belonging to genogroup GII. In a recent report about seasonal AGE in Belgium over the course of ten years, NoVs were detected in 18.7% of all AGE stool samples, and GII.4 predominated them by causing 86.6% of all NoV-positive AGE cases. In this ten-year follow-up, only 3.8% of seasonal NoV AGE was caused by genogroup GI NoVs (141). In Finnish RV vaccine trials in 1994-1995 and 1998-2004, almost 5000 stool specimens were collected from young children with AGE. Among these cases, NoV was found in 25% of the samples, genotypes GII.4, GII.7, GII.3, and GII.b being the types seen more frequently than single cases; genotype GI.3 was found in 1-4% cases in 1998-2003; and other genogroup GI NoVs were found occasionally (169).

In AGE outbreaks, the variation of causative NoV types is greater than in seasonal AGE (174-176). In waterborne outbreaks and outbreaks caused by use of contaminated water in the source of the food (as in the case of imported frozen raspberries planted with contaminated water), the causative NoV most often belongs to the GI genogroup (150,154,175-178). In AGE outbreaks in institutional or crowded settings such as elderly homes or cruise ships, the causative virus is often the predominant NoV type circulating in the population, often a variant of GII.4 (176,179,180).

2.3.3 Clinical picture of norovirus gastroenteritis

The incubation period of NoV AGE is 24-30 hours on average, with variation from 12 to 72 hours (99,139,181). The major symptoms are nausea, vomiting with a sudden onset, abdominal cramps, non-bloody diarrhea (135,173,182). Also fever and generalized soreness of the body may occur, reflecting the name "stomach flu" sometimes used (183,184). The symptomatic period of NoV AGE is reported to be

on average 24-48 hours (139,181,185), with a wide variation from asymptomatic infections to chronic symptoms seen immunocompromised persons. However, the studies consider mainly adults, and the symptomatic period in children is often longer than the average mentioned above (184,186,187).

Also asymptomatic NoV infections exist (139,185). In studies from developing countries, 12-49% of children were found to have NoV present in stools while not presenting with clinical symptoms (188,189). In a study from England NoV was found in up to 25% of previously healthy and asymptomatic children under three years of age and in less than 5% of adults (190).

On the other hand, especially in immunocompromised patients or in neonates, NoV infections can be atypical and severe (138,185) and cause also severe intestinal infection, including necrotizing enterocolitis, or systemic and extraintestinal symptoms such as encephalopathy (138,185,191,192). The emergence of a new NoV GII.4 variant causing AGE symptoms with high fever and intestinal hemorrhage in children was reported in Taiwan in 2013 (193).

Also in otherwise healthy children, NoV AGE often is more severe and symptoms prolonged compared to adults (138,184,185,187). It can lead to dehydration and imbalance in bodily salts and a need for hospital treatment, especially in young children. In general, diarrhea is seen more often in children than in adults.

As with AGEs of other etiologies, NoV AGE cannot be reliably distinguished from other viral AGEs by symptoms only in an individual patient (138). Assessed by the 20-point severity score, the NoV AGE cases in children seen in the hospital are often classified as severe (135,194), but, on average, NoV AGE cases are not quite as severe as RV AGE cases (17,135,138).

2.3.4   Transmission and shedding of norovirus

NoVs are highly transmissible. It has been estimated that the infectious dose may be as low as 18 viral particles (138,195). In a symptomatic person, the viral load in stools may peak two to five days after infection at as much as 10^{11} viruses per gram (139,195). Fecal-oral spread from person to person or via contaminated surfaces is the most important route of transmission (130,135,138,185). The concentration of viral particles is also high in fomites, and they can spread by droplets (196). As NoVs can also be transmitted by aerosols generated by vomiting or flushing the toilet and spread outside a patient's room, the airborne route of transmission is also

possible (139,196,197). NoVs are very stable in the environment and resilient to chemical disinfection substances (37). They survive in aerosols (196) and can survive in a viable form on surfaces for about two weeks and more than two months in water (139,198,199). Transmission often occurs through contaminated food or water, as mentioned above in 2.3.2.2. Regardless the route of transmission, the viruses always have to reach the gastrointestinal tract to reproduce.

It is not yet clear how long the shedding of viable viruses can continue or what the significance is of prolonged shedding in NoV epidemiology. Even in healthy adults, NoVs can be detected in stools for an average of four weeks after an AGE episode (139,163). In small children, elderly people and immunocompromised persons, the shedding can last for much longer (200), even more than a year (164,185). However, asymptomatic shedding seems to be much less contagious than shedding in symptomatic patients (164,185,201), and its significance in transmitting NoV should be studied further. In addition, the window of time in which detectable viruses are found in stools is not automatically equal to the time of shedding of contagious viruses; sensitive PCR methods can also detect RNA of non-viable viruses in stools (202).

2.3.5   Immunity

Protective immunity against NoV is very complex and only partly understood. Virus-specific mucosal and serum IgA antibodies correlate with protection against infection (203,204). The presence of NoVs also stimulates CD4^{+} and CD8^{+} T cells, of which CD4^{+} cells seem to be significant in immunity in re-challenges (205,206). Also VP2-regulated maturation of antigen-presenting cells and VP1-mediated regulation of cytokine induction participate in the induction of protective immunity (205).

In early challenge studies, it was found that protective immunity against re-infection by NoV was short-lived, on the scale of weeks or months (207,208), and cross-protection against different NoV strains did not develop. In contrast, Simmons et al. estimated by mathematic model that immunity against NoV AGE lasts for four to eight years (209). However, in this model, the histo-blood group antigen (HBGA) receptor -linked immunity (see below) was not taken into account. It has also been reported that the levels of NoV-specific antibodies do not correlate with protection, but persons with high antibody levels might be more prone to NoV infection (210-212). Zhu et al. demonstrated that induction and

duration of protective immunity against NoV infections is not consistent, but varies across different NoV strains, clusters and genogroups (205).

The frequency of asymptomatic NoV infections seems to be higher in children with high levels of exposure (213). The role of this phenomenon in NoV epidemiology is not clear; asymptomatic infections do not usually seem to easily transmit the infection.

There are people who do not tend to develop clinical illness after exposure to NoV. This phenomenon is associated to host genotype of HBGA receptors, including H type, ABO blood group and Lewis antigens in the gut epithelium. NoVs recognize HBGA receptors in binding to gastroduodenal epithelial cells. Mutations, which lead to lack of expression of HBGAs on the surface of intestinal cells, have been associated with resistance to NoV infections. About 20% of Europeans do not have HBGAs in the gut epithelium and are not as prone to NoV infections as the rest (139,210,214).

2.3.6   Diagnosis of norovirus gastroenteritis

In the 1970s, the method for detecting NoVs was EM or immune-EM (14). Antigen-detecting tests using monoclonal antibodies have not proven to be ideal in detecting NoVs (215-217). Challenges in antigen testing include the high antigenic variety and constant evolution in NoV strains and the high viral load needed for a positive test signal in ELISA.

Today, NoVs are detected from stools or, less commonly, from fomites or food and environmental samples by RT-PCR. The virus genogroup, genotype and variant can be detected by further sequencing the partial genome. In the 1990s, several primer pairs were needed for detection, and the technique was laborious (218). In the 2000s, detection has based on primers targeting a conserved region of the genome open reading frame 1 (ORF1), which codes the viral RNA polymerase (219). The NoV genome ORF2 region C coding viral capsid can be used in determining the virus genotype and variant. Nowadays it is recommended that both polymerase and capsid genotypes are determined, because recombination is common (134).

Lately, rapid multiplex PCR tests for diarrheal pathogens have been developed and become commercially available. In the future, it might be reasonable to expect detection of the causative agent of AGE with less strict indications and to gain more information about the epidemiology of AGE in both children and adults.

In clinical practice, the purpose is to find the causative agent for the individual patient. For epidemiological reasons, determining the cause of an outbreak, a few stool samples (three to five) are usually studied. In evaluating NoV as the causative agent of AGE outbreak without viral laboratory tests, Kaplan has presented the following clinical criteria (182) for adults: 1) vomiting in more than half of patients, 2) an average incubation time of 24-48 hours, 3) an average duration of illness of 12-60 h, and 4) the absence of bacterial pathogens in stool culture. The criteria have been evaluated to be highly specific in clinical practice for adults (220).

2.3.7 Significance

The predomination of NoVs as the causative agent of AGE has lately been shown in several studies (27,137,141,221-225). In the parts of the world where RV is not yet diminished by universal vaccination, the most important AGE causative agent in children is still RV, with NoVs in the second place. In the countries where RV vaccines have been adopted with high coverage, the NoVs have become the major AGE causative agents, also in children (27,223,224,226,227), because the RV vaccination does not affect the incidence of NoVs.

2.3.7.1 Morbidity and mortality

NoVs are major cause of AGE worldwide, causing enormous morbidity. It is estimated that NoVs cause 200 000 deaths in the world every year, most of them in developing countries (144). In high-income countries such as Finland, deaths due to NoV AGE are rare, but severe infections, particularly in infants and the elderly, are frequently seen. These infections cause hospitalizations and lost work days and thus impose substantial financial costs besides illness and sick leave days.

In United States, it has been estimated that NoVs annually cause 570-800 deaths, 56 000-71 000 hospitalizations, 400 000 emergency department visits, 1.7-1.9 million outpatient visits, and total of 19-21 million AGE episodes there (221). In a recent European study it was estimated that NoV annually causes 102 deaths, 37 000-53 000 hospitalizations, 296 000-800 000 medical consultations, and 1.2-5.7 million episodes in children less than five years of age in Europe (227). The wide range of the estimates suggests the difficulties in evaluating the real NoV AGE burden.

In a meta-analysis of published studies on the global prevalence of NoV AGE, the pooled prevalence of NoV in AGE cases was calculated to be 18%; in community the NoV AGE prevalence was 24%, in outpatient cases 20%, and in inpatient cases 17%. The prevalence was the same in all age groups: both children and adults (136).

Already before the NoVs were recognized as important causative agents in seasonal AGE, they were known as the major causative agents in waterborne and foodborne AGE outbreaks. There are several internet-based surveillance systems for NoV outbreaks, e.g. NoroNet (members in Europe, Australia and Asia) and CaliciNet (US), which also serve to follow up on the NoV genotypes. However, estimating the incidence of NoV AGE outbreaks is at least as difficult as estimating the burden of seasonal AGE. Besides seasonal AGE and outbreaks, probably also a substantial part of travelers' diarrhea and "food poisoning" cases are in fact NoV infections.

2.3.7.2   Economic significance

The cost to society of NoV infections is difficult to estimate, because the majority of AGE episodes are not diagnosed and are treated at home. Furthermore, NoVs cause the majority of outbreaks, of which the frequency and extent cannot be predicted or extrapolated to other periods of time or to larger populations.

The societal costs of NoV AGE in children <5 years of age has been estimated in the United States. The average cost per hospitalized case was $3918, per emergency department treatment $435, and per outpatient visit $151. Based on these figures and the average number of NoV AGE cases in United States, the total direct health care costs totaled more than $273 million (223). The indirect costs, for example from lost working days, and the out-of-pocket costs for the families were not included.

Besides treatment costs, AGE outbreaks in health care settings result in massive indirect costs due to lost resources (lost working days of infected nursing staff and bed and ward closures causing lost treatment resources) and fighting the epidemic by other means.

2.4 Other causative agents of viral gastroenteritis in children

2.4.1 Sapovirus

Sapoviruses (SaVs) were former known as Sapporo-like viruses. They are small, single-stranded RNA viruses belonging to the HuCV family. Four genogroups of the genus SaV are known to cause AGE in humans: GI, GII, GIV and GV. The genogroups can further be divided into genotypes, of which at least 16 are identified (228,229).

SaVs cause AGE mainly in young children (28,229), but are also known as a cause of AGE outbreaks (230-232). In surveillances of the causative agents of AGE in children, SaVs have been detected in 0-19% of the cases (28,233-239).

Like NoVs, SaVs cannot be cultured, and their prevalence or significance as not recognized until sensitive RT-PCR techniques became available. However, in many research laboratories like our laboratory, SaVs are found along with NoVs by using "Calicivirus RT-PCR," which detects both NoVs and SaVs.

2.4.2 Adenovirus

Adenoviruses (AdVs) are double-stranded DNA viruses. They were first seen in diarrheal stool samples by electron microscopy in 1975 (240). AdVs cause different clinical infections depending on the virus (110,241), including respiratory tract infections, unspecific fever and AGE symptoms.

Enteric AdVs, serotypes 40 and 41, are known to cause both seasonal AGE and AGE outbreaks in children and adults. In most studies, AdVs are reported to cause 4-10% of the seasonal AGE in children (26,226,237,242,243). However, in a Swedish study from the 1980s, the share of AdVs in 416 pediatric AGE cases was 13.5%. Most of the children were less than 12 months of age in this study (241).

The major symptoms of AdV gastroenteritis are diarrhea, fever and vomiting, often associated with stomachache (241,243), but the severity of AdV AGE episodes is usually less than that of RV AGE (243). In AdV AGE, cases are seen evenly throughout a year (241,242), unlike RVs and NoVs.

AdVs can be detected from stool samples by ELISA or PCR (241,244,245). The commercially available AdV ELISA test works well and is commonly used in

clinical practice. For study purposes, the PCR method is preferable because ELISA also detects nonenteric AdVs (243).

2.4.3   Astrovirus

Human astroviruses are single-stranded RNA viruses. Eight serotypes are known (246). Also these viruses were found by electron microscopy in 1975, by two independent research groups (247,248). In studies, astroviruses have been detected in less than 10% of all viral AGE in children (26,246,249). On the other hand, most children are seropositive against astroviruses by five years of age (246,250). This supports the finding that, compared to RVs, HuCVs and AdVs, astroviruses cause milder illness, and patients are not often referred to medical care.

Typical symptoms are mild watery diarrhea with or without vomiting, stomachache and fever (246). Astroviruses are very rarely reported in immunocompetent adults (246), perhaps because of protective antibodies from past infection, or just because the infection is mild enough not to result in medical consultation.

2.4.4   Other viruses

Aichiviruses (AiV) have most often been associated with foodborne AGE, usually caused by contaminated seafood (251,252). AiV is usually not the primary causative agent of AGE, but it is often found in mixed AGE with other enteropathogen(s).

AiV was first reported in Finnish children from the RV vaccine study material (253), in which five AiV cases were found out of 1063 (0.5%). Of these cases, four were co-infections.

Human bocaviruses (HBoVs) (254-259) and human coronaviruses (HCoVs) (260), which are primarily respiratory pathogens and can be detected from respiratory samples during infection, have sometimes been also associated with AGE in children. However, it seems that they are not significant AGE pathogens, even if they can be found in stools from children with AGE symptoms: usually the children are also presenting with respiratory symptoms (261-263).

2.5 Vaccines against acute viral gastroenteritis

2.5.1 Rotavirus vaccines

The RV vaccines in clinical use are orally administered live vaccines with reassorted human-animal RVs or attenuated human RVs. The efficacy of these vaccines relies upon heterotypic cross-immunity: after a natural or introduced infection caused by one RV type, the induced immunity protects against consecutive RV infections, and after two infections the protective immunity against moderate to severe RV AGE – also against heterotypic RVs – is excellent and long-lasting (59,105-107).

Of the five most common RV genotypes in Western countries (G1P[8], G9P[8], G2P[4], G3P[8] and G4P[8]), the G2P[4] genotype has been suspected of an ability to escape vaccine-induced cross-immunity better than the other common RV genotypes (107,264,265). However, RV vaccines still show efficacy against severe AGE associated with G2P[4] (266).

2.5.1.1 Rotashield®: rhesus rotavirus vaccine

The first vaccine against RV that became commercially available was derived from rhesus monkey RV G3P[3] reassorted with human VP7 antigens G1, G2 and G3. The full vaccine series consisted of three doses of oral live vaccine scheduled at two, four and six months of age. The vaccine was highly reactogenic and induced febrile reactions in many vaccinees (116).

This vaccine, RRV-TV (tetravalent rhesus RV) or Rotashield®, was launched in the USA in 1998. It was recommended in the routine immunization of children on the above-mentioned schedule, and a catch-up program of children up to nine months of age in the first year (267). About 600 000 children did enter the immunization program until the vaccine was withdrawn in 1999 due to association with vaccination and intestinal intussusception. After withdrawal, in further studies, the relative risk of intussusception following vaccination was assessed to be 58.9 (268), or between 1/10 000 and 1/32 000 vaccinated children. The risk was associated with the age of the vaccinated child: if the first dose of vaccine was given at between three and nine months of age, as in the catch-up schedule, the risk of intussusception was elevated (269,270).

The observed efficacy of Rotashield® against severe rotavirus AGE was 82-91% against severe AGE, and efficacy against any RV AGE was 57-66% (117,271,272).

2.5.1.2   Rotateq®: bovine-human reassorted rotavirus vaccine

Shadowed by Rotashield®, the second generation of RV vaccines were exposed to extensive preclinical trials before entering clinical practice.

Rotateq® is a five-component human-bovine reassortant rotavirus vaccine in which human RV VP7 capsid proteins G1, G2, G3 and G4 are reassorted with bovine virus expressing P7[5], and one human VP4 P-type P[8] reassorted with bovine VP7 G6 (116,264,273,274). The result is "pentavalent" (RV5) vaccine. The full vaccination series consists of three doses. The first dose is given between six and twelve weeks of age, and the subsequent doses at 4-10-week intervals until the third dose is administered before 32 weeks of age. Rotateq® had an efficacy of 100% against severe AGE and about 73% efficacy against RV AGE of any severity in Finland (33).

The RV5 vaccine Rotateq® became available in Finland in 2006, and it was introduced in the Finnish national vaccination program in July 2009. In between, the vaccine was commercially available, and the vaccination coverage in the target population reached about 6%. Now, six years after implementing Rotateq® in the national program, the vaccination coverage in the child population is about 91%, and practically all the RV vaccine administered is Rotateq® (125).

Diarrhea, vomiting and low-grade fever associated with vaccination with RV5 have been reported at a similar or slightly higher rate in vaccinees and in placebo recipients (264,275). More severe diarrhea associated with the emergence of a human-bovine double reassortant RV in vaccinees has been seen in case reports (276-278). The major adverse effect of interest, intussusception, has been continuously studied. A small risk has been found of intussusception associated with days 3-7 after the first dose of RV vaccine. The relative risk of intussusception associated with RV5 has been between 2.9 and 9.9 after the first dose, accounting for 0.79-7.0 excess cases of intussusception per 100 000 children (268,279-283).

2.5.1.3   Rotarix®: human attenuated rotavirus vaccine

The other currently available vaccine against RV is also an orally administered live vaccine. Rotarix® has been attenuated from human RV-type G1P[8], and it is a "monovalent" vaccine (RV1). The vaccination consists of two doses given between 6 and 24 weeks of age with at least four-week intervals between doses. Adverse effects associated with RV1 have been sparse. Irritability and vomiting have been seen, but no significant association with fever reactions (284). Recently, a similar slightly elevated risk of intussusception following the first dose of RV1 vaccine than with RV5 has been noted, the relative risk being between 1.1 and 6.8 (268,282,285), accounting for 1.2 to 2.8 per 100 000 children after the first dose of RV1 (283,286).

   The efficacy of RV1 against severe rotavirus AGE has been about 90% and against all AGE between 58% and 90%, with the lowest efficacy against G2P[4]. (265,266).

   Also Rotarix® became commercially available in Finland in 2006, and between 2006 and 2009 its coverage reached about 29% of the target population. However, the use of Rotarix® has been minimal since Rotateq® was chosen for the Finnish national immunization program in 2009, and the former is no longer available in Finland.

2.5.2   The effectiveness and impact of rotavirus vaccination

The RV5 vaccine was introduced in the national immunization program (NIP) of Finland in September 2009. Since then, about 95% of Finnish newborn children have been immunized against RV. In this study, we first described the period when the vaccines became available and vaccination coverage was on the rise, but the universal vaccination program had not been launched. After 2009, the RV AGE decreased among the children of the target population (287-289), and the effect can be clearly seen in hospital wards treating infectious diseases of children.

   Besides Finland, a remarkable reduction of RV AGE has been seen everywhere where the RV vaccine has been adopted, with the vaccine effectiveness (reported most often for the RV AGE hospitalizations) depending on the vaccine coverage rate in the area (287,290-293). In most studies, the reduction in hospitalizations for RV AGE has been used to calculate RV vaccine effectiveness.

   The US was among the first countries to adopt RV vaccination in its NIP in 2006. The RV vaccine was reported to have reduced RV AGE hospitalizations by

87-96% in three US counties in 2006–2009 (294). The effect was noted as early as in the first RV season after introduction of the RV vaccine, with 16% reduction in hospitalizations, and there was a 45% reduction in the second season (295). In further follow-up in 2009–2011, the effectiveness of RV5 on the combined number of hospitalizations and emergency department visits was 84%, and the effectiveness of RV1 was 70% in children less than five years of age (296). In Australia, the reduction of RV AGE cases was reported to be 66-77% (297,298). An important and very promising report about the impact of RV vaccines on childhood mortality comes from Mexico, where the RV G1P[8] vaccine reduced diarrhea-related child mortality by 35%, and this decline has been sustained for at least four years (119).

As of December 2015, RV vaccine has been introduced into the NIPs of 80 countries, including 15 European, 28 African, and 20 American countries (Rotacouncil.org), which are presented in Figure 3.
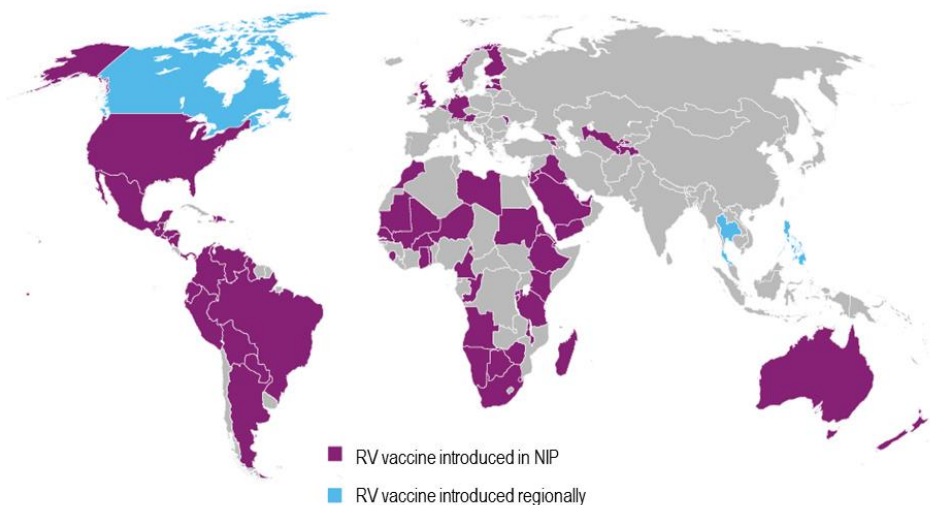
Figure 3. The countries, in which a vaccine against rotavirus (RV) has been introduced in the national immunization programme (NIP). Modified from www.rotacouncil.org.

The RV genotypes included in the RV vaccines available today are G1P[8] in RV1, and G1, G2, G3, G4 and P[8] in RV5. It has been discussed whether the protection achieved by using one of these vaccines is enough, or whether replacement of the circulating RV genotypes towards the non-vaccine types will diminish vaccine efficacy (299). The RV vaccines also seem to offer good

protection against non-vaccine-type RV AGE (76,296,300,301). No remarkable replacement of the genotypes or constant increase in the incidence of G2P[4] has been associated to either of the vaccines in published studies (76,83,87,302,303). In some studies, changes in circulating RV genotypes have been seen (304,305), but in these studies the follow-up periods have been short, and the change has not been greater than natural variation.

Another debated issue is whether the other AGE-causing viruses might have benefited from the decrease in RV AGE, and whether the incidence of other viral AGE might rise. No evidence of replacement of RV AGE with AGE caused by other viruses has been seen, but, as expected, the RV vaccines have not influenced the incidence of other-cause AGE, neither for the better nor for the worse (303).

After the RV vaccines became available, the estimated mortality and overall burden for RV AGE has decreased. In a recent estimation, RV was assessed to cause 180 000-450 000 deaths, 25 million clinic visits and two million hospital admissions in children under five years of age every year on a global basis (306).

In developed countries, the vaccines against RVs have already changed the rates of childhood morbidity in the countries that have adopted the vaccine. Effectiveness data in Africa are only emerging at this time.

2.5.3   Other considerations about rotavirus vaccines

Both of the RV vaccines are orally administered live vaccines. It is essential for the vaccine viruses to enter the intestines and reproduce in intestine mucosal cells in order to induce immunogenic reactions. Vaccine viruses should not induce clinical symptoms of AGE. The vaccine type viruses are excreted into stools of vaccinated children (278,307) and may be transmitted to other children. There has been concern about whether immunocompromised children (or adults) when exposed to vaccine viruses, could become acutely or chronically infected; or whether RV vaccine virus in immunocompromised host could be reassorted and become enteropathogenic again. Viral reassortation occurs (277,308), but the significance of it needs further surveillance.

Immunodeficiency is a contraindication for live vaccines, but the immunodeficiency may not yet be known in the target group. RV vaccine given to children with a severe combined immunodeficiency has led to prolonged diarrhea and virus shedding (309-311). By far, however, really severe complications have not been reported associated with exposure to the vaccine viruses indirectly, via other

vaccinated children. Studies of the safety of RV vaccines in HIV-infected adults (312) and children (313,314) have been published, and no severe adverse effects were reported.

2.5.4   Norovirus vaccine research

A promising approach in NoV vaccine research is using nonproductive virus-like proteins (VLPs) as antigen compounds. These VLPs consist of the NoV capsid protein VP1, which can be produced by recombinant baculoviruses harvested in insect cells. VP1 forms empty NoV capsids that are morphologically and antigenetically similar to the native NoV virions and highly immunogenic (132,315,316).

3　Aims of the study

This study of children treated for AGE at the Tampere University Hospital has the following objectives:

1) To study the role of RV and NoV as causative agents of AGE in children at the time of introduction of RV vaccination in Finland.
2) To describe the clinical features and overall severity of RV and NoV AGE in children.
3) To examine the genotypes of RVs and NoVs circulating in children.
4) To estimate the costs of RV and NoV AGE to families and society.
5) To describe the causative agents and clinical features of AGE resulting from a waterborne outbreak of AGE in Pirkanmaa in children.
6) To use this epidemiological study as a baseline to investigate the early effect of the universal RV vaccination program on AGE seen at the Tampere University Hospital.

4 Materials and methods

4.1 Study materials (patients)

The study was approved by the Ethics Committee of The Pirkanmaa Hospital District.

All children ≤15 years of age at the Tampere University Hospital seen either in the emergency room or admitted to a hospital ward with AGE were eligible for enrolment. The dedicated study nurse mostly handled recruiting the children for our study in the daytime, and a nurse in charge in an outpatient clinic or hospital ward at other times. Prior to enrolment, a parent or legal guardian signed an informed consent.

The parents were interviewed about the child's AGE symptoms and rotavirus vaccination status. They were also asked to complete a questionnaire after recovery about the total duration of the AGE symptoms. The families of hospitalized children were asked to participate also in the study of the costs of AGE, and the participating families were interviewed in the ward and again after discharge (see 4.2.3.1).

After discharge from the emergency room (ER) or the hospital ward, the clinical information and test results of the AGE episode were collected from the hospital's documents. A stool sample was collected in ER or in the hospital ward. If the child did not pass stools while in hospital, no stool sample was obtained.

If the child had more than one ER visit or hospitalization during the study period, we deemed the AGE symptoms to belong to the same episode if there were less than seven symptom-free days in between. Otherwise, the visits were considered to represent two separate episodes.

The information on all the AGE patients ≤ 15 years of age seen at the hospital during the study period was collected from the hospital's database. For this purpose, all the ICD-10 diagnoses of the groups A01-A09 were retrieved.

In the study period from September 2006 to August 2008, a total of 1723 children ≤15 years were seen at the Tampere University Hospital because of AGE. Out of these, 1193 children were recruited to the study. The reasons for non-recruitment were either unwillingness to participate or hospital staff not being able
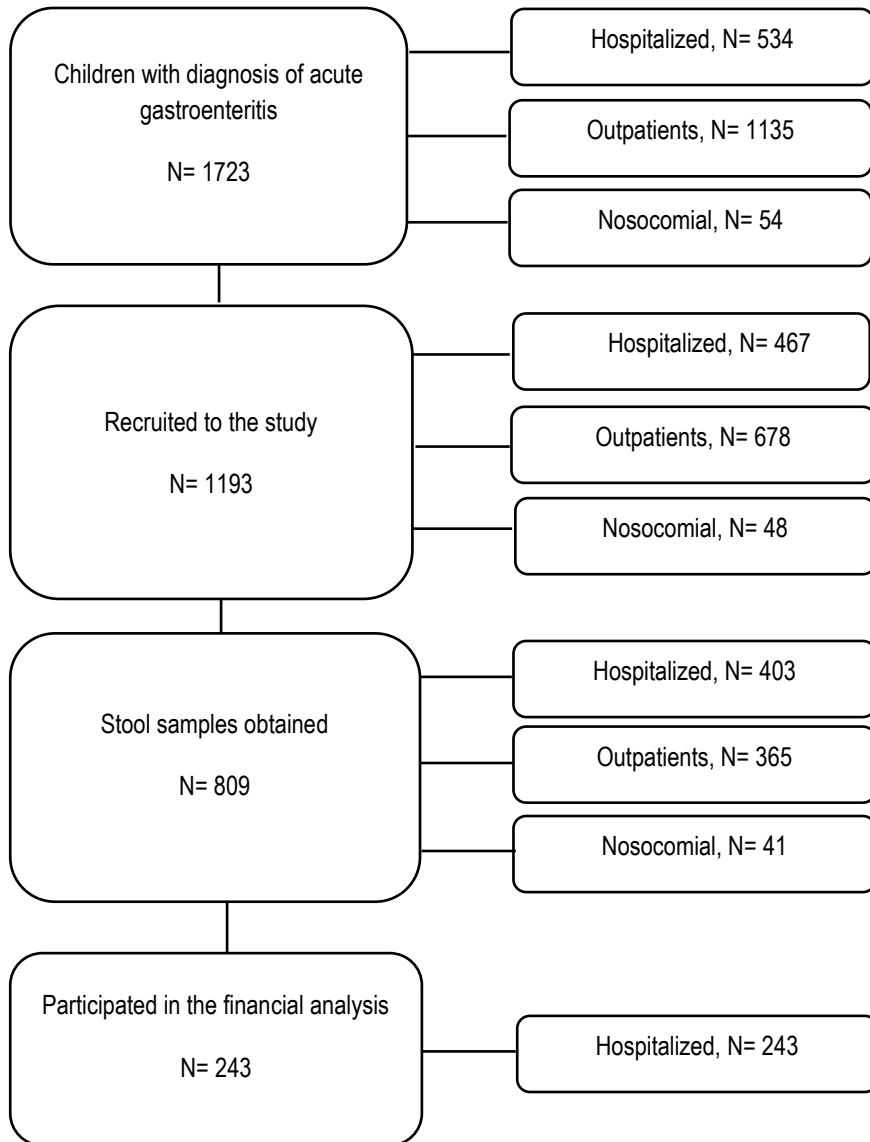
Figure 4. Flow chart of the study recruitment of the children seen because of acute gastroenteritis in the two-year study period, September 2006 – August 2008.

to recruit in the clinic or the ward, often due to lack of time. Of the 1193 children recruited with AGE, a stool sample was collected in 809 (68%) cases (Figure 4); the remainder did not pass stools during their stay at the hospital. The number of

AGE cases with stool samples was 341 in the first year (September 2006 to August 2007), and 468 in the second (September 2007 to August 2008).

Study **I** reports the large Pirkanmaa waterborne AGE outbreak in the town of Nokia, which took place during the study period, in November - December 2007. The outbreak was caused by contamination of drinking water with sewage water as a result of a pipe connecting these two water lines being accidentally left open. It was estimated that about 6500 people caught AGE in connection with this contamination, and a total of 1222 patients were seen in primary health care (151). Of these, all the children who needed pediatric consultation were referred to Tampere University Hospital and the cases were recorded. The cases from this outbreak are included in Study **II,** which discusses RV epidemiology. However, we decided to leave these cases out of Study **III**, which compares the severity of NoV AGE and RV AGE and NoV epidemiology, because of the unusual clinical features of these cases, which could skew the normal distribution of severity.

After the first two-year study period, the follow-up was continued in September 2009 – August 2011. The enrollment methods were similar to those of the study period in 2006–2008 described above. The number of enrolled children in the latter period was 495, and 330 (from 66% of the recruited) stool samples were obtained: 160 in 2009–2010 and 170 in 2010–2011. Of these 330 children, 144 (44%) were treated as outpatients and 186 (56%) were hospitalized. Study **IV** reports the results of this continued follow-up period.

4.2   Clinical and statistical methods

4.2.1   Assessing the severity

Overall severity of the AGE episodes was assessed by the 20-point score or "Vesikari-score" (34), which considers the intensity and duration of diarrhea and vomiting, degree of fever and dehydration, and the treatment needed. With this scale, the AGE episode is considered severe if it receives $\geq 11/20$ points, and moderately severe if the severity score is 7-10 points.

The severity of those AGE episodes, from which all the essential information was available, was assessed either by interviews or by retrieving the information from the clinical records. In practice, the majority of these cases were treated in the

hospital ward: the outpatient clinic staff enrolled many of the children treated as outpatients, and often not all the data had been collected.

The Mann-Whitney U test was used in comparing the statistical significance of the difference between symptoms and severity scores of AGE episodes caused by different causative agents. The analysis was performed using version 22.0 of IBM SPSS Statistics software (SPSS Inc., Chicago, IL).

4.2.2   Assessing the coverage of rotavirus vaccine

We received open-access data on the number of the RV vaccine doses sold in Finland during the September 2006 – August 2008 study period from the vaccine-producing medical companies GlaxoSmithKline and Sanofi Pasteur MSD. From the monthly number of vaccine doses sold, we estimated the number of children who had received all the vaccine doses of either vaccine, and calculated the coverage of RV vaccination per birth cohort in each year and each season. Data about potential variation in local vaccination coverage levels was not available. The birth cohort size was based on the number of live births, and that data was obtained from Statistics Finland.

Based on the sales records data for these vaccines and the size of the target population in Finland, the coverage of RV vaccines in the target population was 22% in the first follow-up year and 35% in the second. By the second year, 29% of the vaccine doses used were the G1P[8] vaccine (RV1), Rotarix®, and 6% were the pentavalent G1-4,P[8] vaccine (RV5), Rotateq®.

The information about the coverage of RV vaccine in the target population after the RV vaccine was included in the NIP, i.e. in 2009–2011, was assessed from THL.

4.2.3   The financial burden of acute gastroenteritis

4.2.3.1   Cost to the families

Evaluation of the financial burden on the individual families caused by RV AGE and NoV AGE was based on interviews. The families of the children recruited to the study and hospitalized were invited to take part in this survey, and the parents of 243 children participated (Figure 4). Stool samples from all these children were

obtained. We interviewed, using structured questionnaire, these parents first in the hospital ward during the acute treatment about the costs they had incurred up to that moment, and the parents were given a questionnaire for keeping track of costs after discharge. The date of a follow-up call was agreed on: the parents were telephoned by the study nurse or the researcher about two weeks after the first interview in the ward, and they reported the costs incurred after discharge and the total number of lost working days.

We evaluated the total average out-of-pocket cost for one AGE episode by putting together the averages of the different expense categories, the percentage of families who reported their expenses and the median of all the expenses reported. The median was used because it describes the average better than the mean: there were a few families who reported very high expenses, which pulled the mean value up.

4.2.3.2   Costs for the society

From the information provided by the Tampere University Hospital and the City of Tampere, we estimated the societal costs based on what the hospital charged communities for treating AGE patients in the hospital ward or outpatient clinic, and on the real expenses resulting from utilizing primary health care resources.

In evaluating the financial burden of RV and NoV AGE at the primary health care level, we compared our results with the assumptions made by THL (118). We extrapolated the ratio of RV AGE cases treated in primary care, in outpatient clinics and hospitalized to the RV AGE results of our study. Furthermore, we made an assumption that, in NoV AGE, the ratio of AGE cases treated in primary care is the same as those treated as outpatients in hospital, which probably underestimates the amount of NoV AGE in primary care. These numbers of RV and NoV AGE were extrapolated to the child population of Finland.

4.3   Laboratory methods

4.3.1   Method for detecting the rotaviruses

All the stool samples were studied for RV by reverse-transcription (RT) PCR, and most samples also by ELISA. ELISA was not performed if the stool specimen was

diffused in a diaper or if the amount of specimen was too small for RV (and HuCV) PCR and ELISA. RV G-types were determined by RT-PCR as described by Pang et al. (17) with the Taq polymerase replaced by GoTaq® polymerase (Promega, Madison, WI, USA). RV G-types were determined using two different primer sets introduced by Gouvea et al. (113) and Das et al. (317). To confirm the G-type results, all the RV VP7 amplicons were sequenced. The RV P-types were determined by RT-PCR amplifying the VP4 genome segment, using a method modified from that of Simmonds et al. (318). Reverse primers in the second-round PCR reaction were the same for P[4] and P [8], but for P[6] and the forward primer, modified primers were used (5'- GATGGTCCDTATCARCC-3' for VP4fwd, and 5'- ATTTGAAGTTGACGAGTA-3' for P[6]). This method detects P-types P[4], P[6] and P[8].

The presence of RV antigen in stools was detected with ELISA using IDEIA® Rotavirus kits (Oxoid Ltd., UK) according to the manufacturer's protocol.

4.3.2 Method for detecting the noroviruses and sapoviruses

The detection method for HuCVs was modified from the RT-PCR method first introduced by Jiang and Farkas (319,320). The samples were tested using the primer mixture p289H,I,IUB (antisense) and p290H,I,J,K,IUB (sense) with the RT-PCR method described earlier (319,320), with a modification described by Puustinen (169). This RT-PCR detects the RNA polymerase region, amplifying a 319 bp amplicon for NoVs and 331 bp amplicon for sapoviruses.

All the NoV positive amplicons from RNA polymerase region were further sequenced in our laboratory to detect the genogroup and genotype.

4.3.3 Other viruses

AdVs were detected by a previously described nested PCR (244) using two primer sets. The presence of AiVs was ascertained after a separate RT reaction by a nested PCR with random primers. The AiV-detecting PCR method was originally developed by Yamashita et al. (321). The HBoVs were detected by a two-step PCR as described by Risku et al. in 2012 (322,323). The HCoVs were detected using the PCR method setup and optimized in the Virology Laboratory of the Tampere University as described by Risku et al. in 2010 (261).

5 Results

5.1 Epidemiology of rotavirus gastroenteritis (II)

Of all AGE cases with stool samples, RV was the causative agent in 128 of 341 (38%) in the first season and in 293 of 468 (63%) cases in the second: a total of 421 RV-positive AGE cases. The first season was a low season with an unexpectedly low rate of RV AGE cases and the second more like a normal RV season.

5.1.1 The rotavirus gastroenteritis cases

Of the 421 RV AGE cases seen at the hospital, 235/421 (56%) were males and 186/421 (44%) were females. Of the RV AGE cases, 179 (43%) were treated as outpatients, 226 (54%) were hospitalized, and 16 (4%) were nosocomially acquired.

The age distribution of the children with RV AGE is shown in Figure 5. Most of the children (233/421, 55%) were between 6 and 24 months of age, and 149/421 (35%) were between 12 and 24 months of age.

RV AGE was also seen in children ≥5 years of age. In this AGE group, there were 37 RV AGE cases seen in the two follow-up years at the hospital: five cases in the 2006–2007 season, and 32 cases in the 2007–2008 season. Of the latter, 12 were associated to the Pirkanmaa outbreak and one was a nosocomial case, but 19 cases were seasonal RV AGE. Altogether, there were 24 seasonal RV AGE cases in children ≥5 years of age. Of these, 18 (75%) needed hospitalization and 6 (25%) were treated as outpatients.
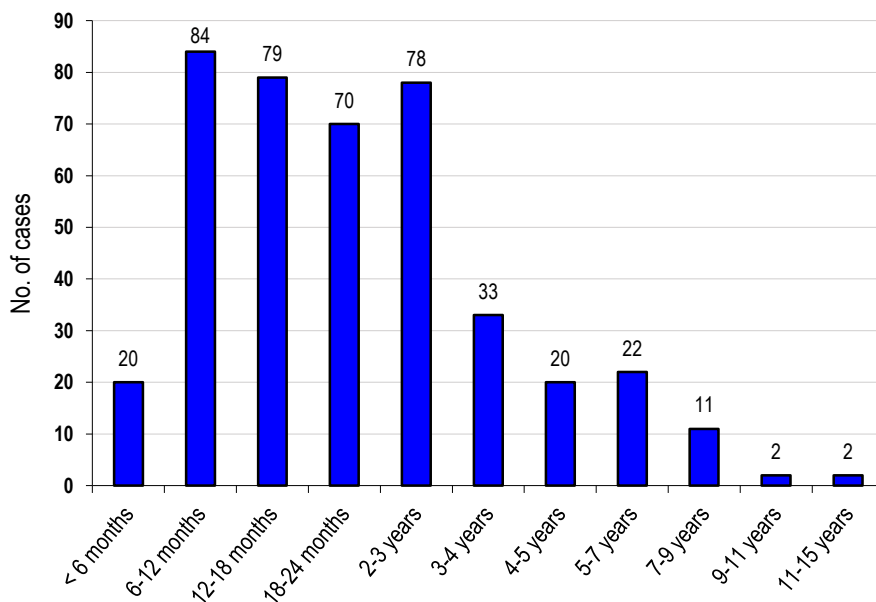
Figure 5. Age of the children diagnosed with rotavirus gastroenteritis in 2006-2008.

5.1.2 Seasonality of rotavirus gastroenteritis

There was a clear seasonality in RV AGE (Figure 6). In the first follow-up year (September 2006 to August 2007), the RV season started late, in January 2007, with the majority of the cases appearing between April and June 2007. The season continued over the spring until as late as July.

In the second follow-up year (September 2007 to August 2008), the RV season started earlier, in October 2007, and continued until June–July 2008. The most active months of the second season were between December 2007 and April 2008. The cases associated to the Pirkanmaa waterborne outbreak are also included in the results of the second year in Study **II.** The outbreak influenced the second AGE season, making the beginning of the RV season very abrupt and bringing on additional cases in December 2007 (Figure 6).

The interval between the RV seasons was shorter than expected as a result of the delayed and then long-lasting first season.
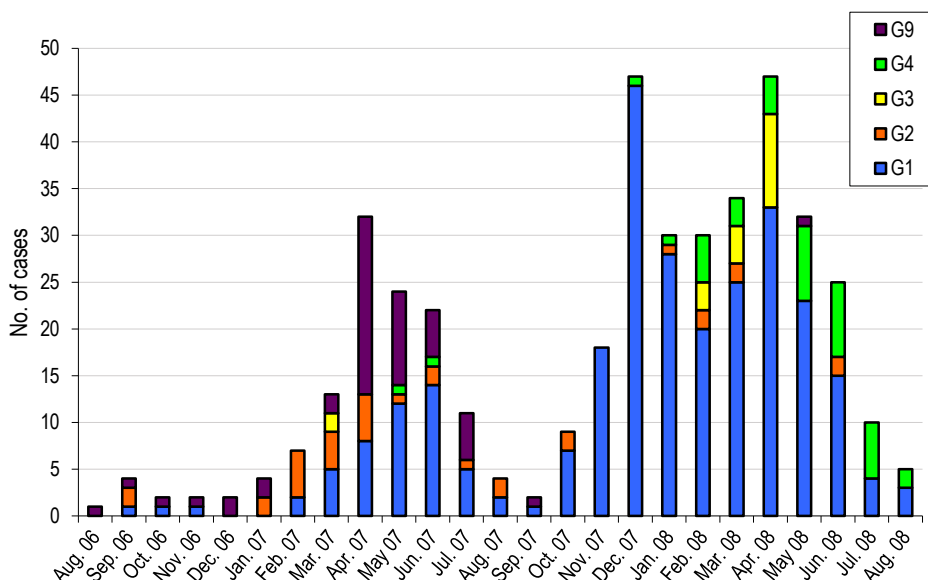
Figure 6. The rotavirus seasons and the detected genotypes by G-type in 2006-2008.

5.1.3   Rotavirus genotypes

In the first follow-up year, which was a low-RV season, the distribution of circulating RV genotypes was different from a typical year (Figure 6). The RV genotypes G1P[8] and G9P[8] were equally common: G1P[8] presenting with 51/128 (39.8%) and G9P[8] presenting with 49/128 (38.3%) of all RV-positive AGE cases. Also RV type G2P[4] was seen commonly, in 24/128 (18.8%) of the RV AGE cases. The other RV genotypes seen were G3P[8] and G4P[8], each with 2/128 (1.6%) of the RV-positive AGE cases.

In the second season, RVs caused the majority (293/468, 63%) of the AGE cases seen at the hospital. The predominating RV genotype was the traditionally dominant G1P[8], with 214/293 (73%) of RV cases. The second most common genotype was G4P[8] with 38/293 (13%) cases, and also G3P[8] was seen with 13 (4%) cases. RV presented genotype G2P[4] in 9/293 (3.1%) cases.

Most of the G and P genotype combinations were the expected: G1, G3, G4 and G9 presented with P[8], and G2 with P[4]. In four cases there were RVs presenting single G genotype G1 with P genotypes P[8] and P[4] found in the same stool sample. Besides the detected genotypes, there were five stool samples (two stool samples with G1 and three with G3), in which the P genotype remained undefined by RT-PCR; and in three stool samples (two with G1 and one with G3) the sample was exhausted and the P type could not be detected. The RV AGE cases seen outside the most active seasons were more often genotypes other than G1 compared to the cases seen during seasons (Figure 6).

The RV AGE cases associated with the Pirkanmaa outbreak (n=33) can be seen in the December 2007 column of Figure 6. All but one of the RV-positive outbreak cases were of genotype G1P[8], one was G4P[8].

5.1.4   Comparison of PCR and ELISA methods in detecting rotaviruses

All the 809 stool samples obtained were studied by RT-PCR, and 631 by ELISA as well. Reasons for not using ELISA were, in 147 cases that the specimen was in a diaper and, in 31 cases that the sample was too small for ELISA testing. All samples that were RV-negative according to PCR testing were also negative according to ELISA. Among the 305 stool samples that were RV-positive according to PCR and could be detected with ELISA, 278 (91%) were also positive according to ELISA.

Of these 27 samples (9%) that were PCR-positive and ELISA-negative, 16 (59%) were of the genotype G1P[8], including one RV1 vaccine-type virus; four (15%) were G2P[4]; four (15%) were G3P[8]; two (7%) were G4P[8]; and one (4%) was G9P[8]. In 15 (56%) of the PCR+/ELISA- cases, a NoV was also found, and a SaV was also found in one case.

5.1.5   Rotavirus gastroenteritis in children who were vaccinated against rotavirus

Among the 1193 enrolled children who were seen at the hospital for AGE in 2006-2008, there were 49 children (4%) who were known to have received at least one dose of one of the RV vaccines available. We obtained stool samples from 36 (73%) of these children.

RV was found in eight (of 36, or 22%) of the stool specimens. In three of these cases, the detected RV was of the vaccine-type G1P[8]; in four cases it was a wild-type G1P[8]; and in one case the RV found belonged to G genotype G3 but the P type could not be detected. In three (of five) of the cases in which a wild-type RVs was found in vaccinated children, a NoV was also found in the samples and was probably the real causative agent of the AGE symptoms (Table 2). Based on these data, the effectiveness of RV1 would have been about 90%.

Out of the total of 36 children who were known to have received vaccine(s) against RV and from whom the stool samples were obtained, NoV was found to be the causative agent of AGE in 14 cases (39%) and SaV in one.

Case	Age in months	1st day of symptoms	Vaccination date(s)	Vaccine	RV genotype	RV-ag EIA	Other pathogens
1	2	5 Sep. 2006	4 Sep. 2006	RV1	G1 Vac	pos.	-
2	4	12 May 2007	9 May 2007	RV1	G1 Vac	neg.	-
3	3	27 Jun. 2007	11 Jun. 2007	RV1	G1 Vac	neg.	-
4	15	2 Dec. 2007	Nov. 06 and Dec. 06	RV1	G1 WT	neg.	NoV GII.4
5	14	11 Apr. 2008	unk	unk	G1	neg.	NoV GII.4
6	11	24 Apr. 2008	unk	unk	G3	neg.	NoV GII.4
7	18	14 Jun. 2008	unk	unk	G1	NT	-
8	27	9 Aug. 2008	unk	unk	G1	pos.	-

Table 2. Information about the children, who were vaccinated against rotavirus and were treated in hospital because of acute gastroenteritis 2006-2008. RV: rotavirus, Vac: vaccine type rotavirus, WT: wild type rotavirus, unk: unknown; NT: not tested, RV-ag EIA: result of rotavirus antigen test.

5.1.6 Effectiveness of rotavirus vaccination

In continuation of the follow-up study in 2006–2008, we studied the epidemiology of RV AGE seen at the same hospital (Tampere University Hospital) in the same age groups (children less than 16 years of age) in 2009–2011 after RV5 was introduced in the NIP in Finland. Between this study and the introduction of universal RV vaccination in September 2009, there was a one-year period in which there was no prospective surveillance.

The coverage of RV vaccines reached 35% among the target population during the study period 2006–2008. After introduction of the vaccine in the NIP, the vaccine coverage was about 93% (among the children born in 2009).

The number of RV AGE cases seen at the hospital, treated as outpatients or hospitalized, declined by 80% from follow-up period in 2006–2008 (421 cases) to the 2009–2011 period (86 cases). Hospitalization was down by 76% (219 in 2006–2008 versus 52 in 2009–2011) and outpatient clinic visits by 81% (177 in 2006–2008 versus 34 in 2009–2011) (Figure 7). The universal RV vaccination has not had an effect on the burden of NoV AGE.
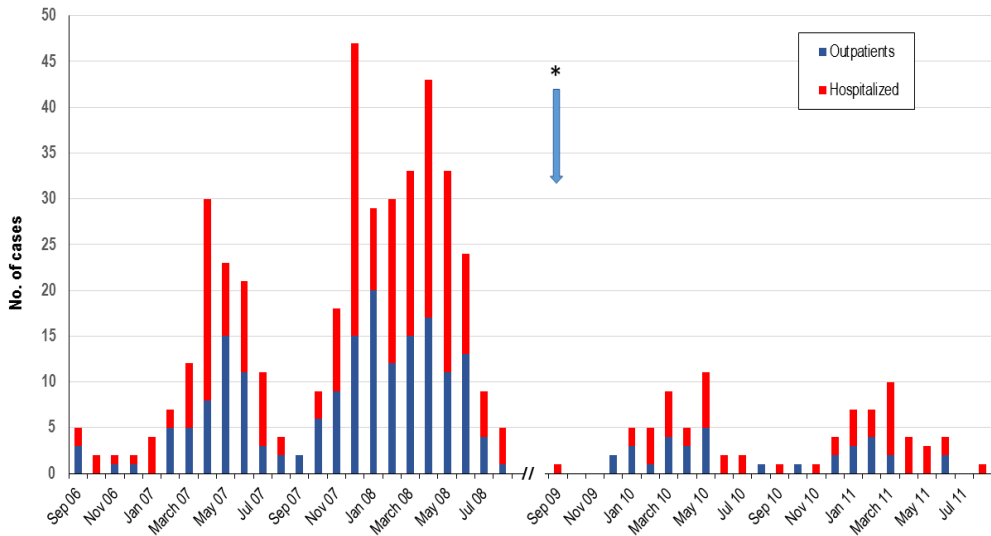
Figure 7. Hospitalizations and outpatient clinic visits because of rotavirus gastroenteritis in children less than 16 years of age in four seasons from September 2006 to August 2011, before and after introducing vaccination against rotavirus in national immunization programme in Finland. *The launch of universal vaccination against rotavirus.

Most of the children seen at the hospital for RV AGE before universal RV vaccination were between 6-24 months of age: 55% (233/421) belonged to these age groups. After the introduction of RV vaccination in the NIP in Finland, in 2009–2011, 36% (31/86) of children belonged to these age groups; most RV AGE cases were seen in children one to three years of age (Figure 8).
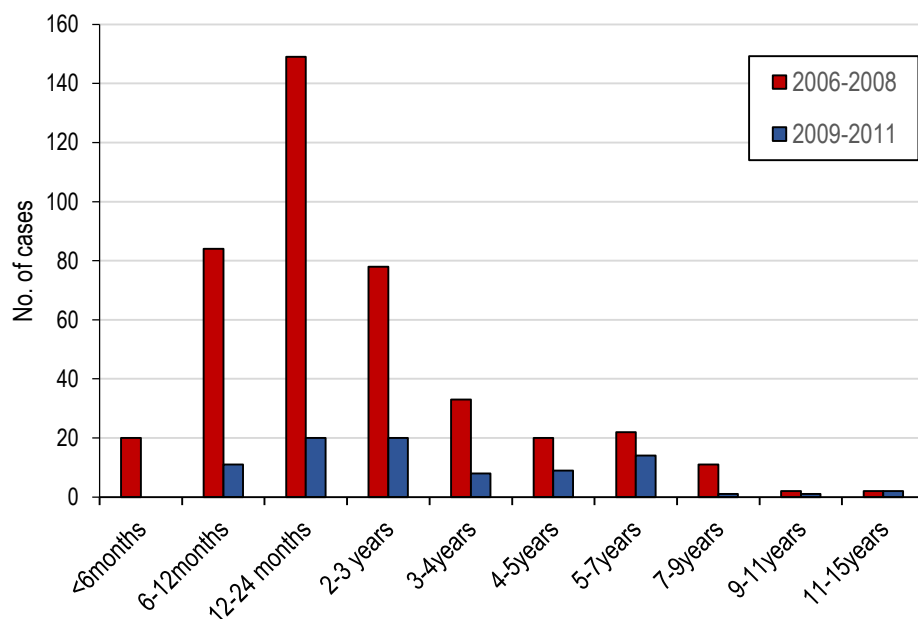
Figure 8. The age distribution of the rotavirus gastroenteritis cases seen at the hospital before the introduction of universal rotavirus vaccination (2006-2008) and after (2009-2011).

5.2 Epidemiology of norovirus gastroenteritis (III)

In the study of seasonal NoV AGE in children, we left the AGE cases associated with the Pirkanmaa waterborne outbreak out of the analysis. These cases are reported in Study **I**. Among these cases, there were 20 NoV positive stool samples.

Thus, 759 cases with stool samples were included: 341 cases from the first AGE season and 418 from the second. NoV was found in 196 of these cases: in 116/341 (34%) of the AGE cases seen at the hospital in the first season and in 80/418 (19%) in the second. Dual infections with NoV and RV detected from the same stool were seen in 11 cases in the first season and in 13 cases in the second, i.e. in a total of 24 (12%) of the NoV positive cases. There were no cases presenting with more than one type of NoV or dual infections presenting with NoV and SaV (Figure 9).

No child was treated at the hospital for more than one episode of NoV AGE during the two follow-up years. There were 15 nosocomial infections among the NoV AGE cases.
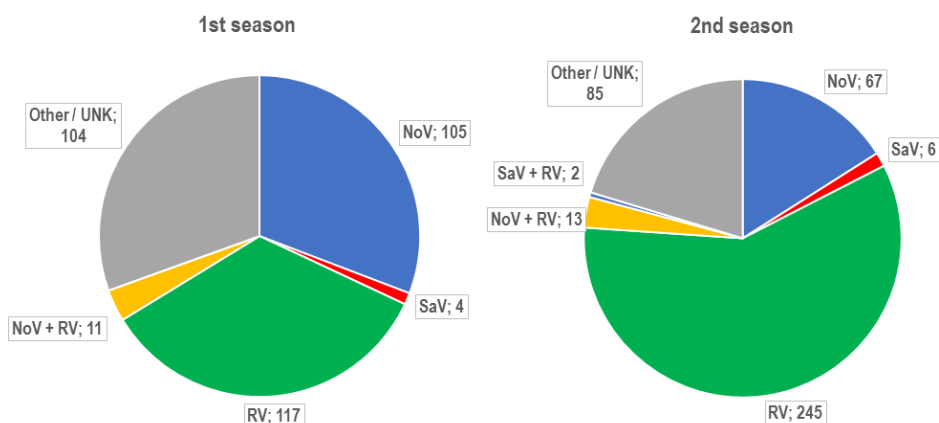
Figure 9. Causative viruses of acute gastroenteritis seen in children at the Tampere University Hospital in the first (September 2006- August 2007) and the second follow-up season (September 2007- August 2008).

5.2.1 The norovirus gastroenteritis cases

Of the total of 196 NoV positive cases, the median age of the children seen at the hospital because of AGE was 15 months, the age range being from 19 days to 13 years and 8 months. Altogether, 72% of the children who were treated because of seasonal NoV AGE were ≤ 24 months of age; or, more precisely, 55% of the cases were between 6-18 months of age. There were also 14 (7%) NoV AGE cases seen in children who were ≥5 years of age, one of which was a nosocomial NoV AGE case (Figure 10).

Of the NoV AGE cases, 117/196 (60%) were male and 79/196 (40%) were female.

The dual infections excluded, of the NoV positive cases in which NoV was the only AGE causative agent found, 81/172 (47%) were treated as outpatients, and 80/172 (47%) were hospitalized. The remaining 11 cases were nosocomially acquired.
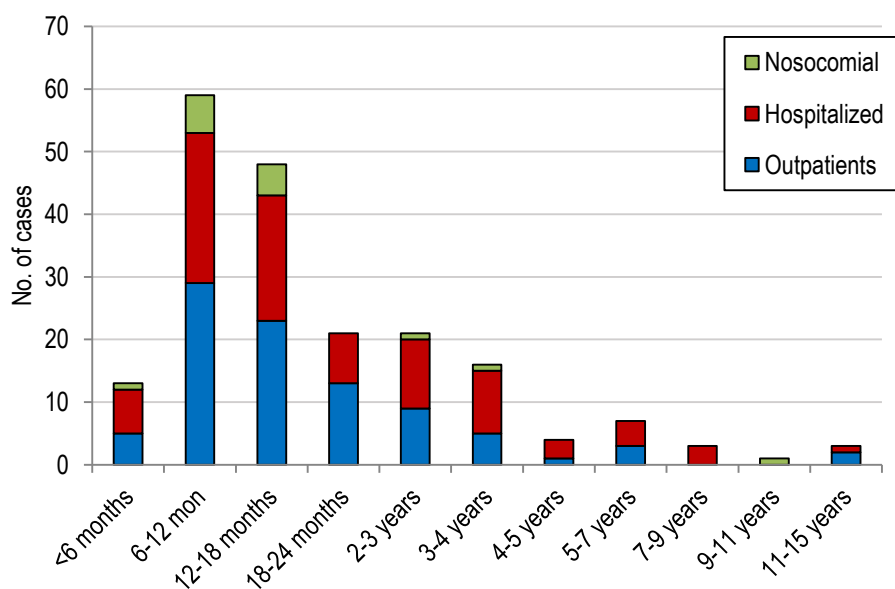
Figure 10. The age and need for treatment in norovirus gastroenteritis cases in 2006-2008.

5.2.2   Seasonality of norovirus gastroenteritis

In the two-year follow-up, a clear seasonality in NoV AGE was observed. The active NoV seasons were during the cold winter months and the periods of low activity in the summer and autumn (Figure 11). In the first follow-up season (September 2006 – August 2007), the highest activity period of NoV AGE was from February to April 2007, and, in the second season (September 2007 – August 2008), the activity level was as highest from January to April 2008, forming a less distinct peak. Between the AGE seasons, no cases of NoV AGE were seen at the hospital in August, September or October 2007.

The seasons were different compared to each other: in the first season the NoV activity overall was high, and the NoV cases seen were almost exclusively of the dominant GII.4 genotype. The second season was not as active, and there was more variation in the NoV genotypes.

5.2.3 Norovirus genotypes

The NoV genotypes seen during the two seasons are shown in Figure 11. In the first AGE season, in which 116 NoV AGE cases were detected, GII.4 was the predominant NoV genotype with 111/116 (96%) cases. The other genotypes seen in the first season were GII.1 (two cases, or 2%), GII.7, GIIc and G1.6 (one case each, or <1%).

In the second follow-up season, excluding the Pirkanmaa-outbreak-associated cases, 80 cases of NoV AGE were detected. The major genotype was again GII.4, with 64/80 cases (80%), followed by GIIb (11/80, 14%), GII.7 (4/80, 5%) and GII.2 (1/80, 1%).

After publication of Study III, the NoV positive stool specimens were reanalyzed using the capsid-PCR method. Some of the detections were clarified: the NoV genotypes first reported as "GII.b" were found to belong to genotype GII.3, and the NoV reported as "GII.c" was found to belong to genotype GII.7 (324).
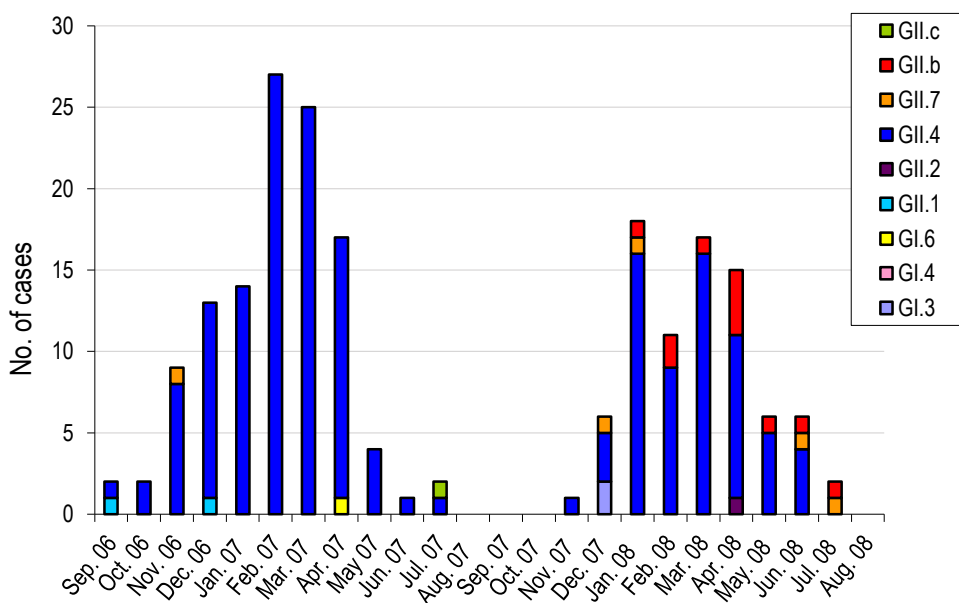
Figure 11. Norovirus genotypes seen in children between September 2006 and August 2008.

5.2.4   The 2009-2011 norovirus gastroenteritis cases

In the follow-up period of 2009–2011, when 330 stool samples were obtained from children with AGE, 112 (34%) were positive for NoV: 53/160 (33%) in 2009–2010 and 59/170 (35%) in 2010–2011. The age of the children with NoV AGE in this period was similar to the 2006–2008 cases: the range was seven days to 15 years, with a median of 12 months. In most of the cases (84/111, 76%) the children were younger than 24 months.

Of the 112 NoV positive AGE cases, 72 (65%) were of genotype GII.4: 37/52 (71%) in 2009–2010 and 35/59 (59%) in 2010–2011. Other genotypes seen were GII.b (n=15, 14%), GII.7 (n=14, 13%), GII.g (n=6, 5%), GI.4 (n=2, 2%), GI.3 (n=1, 1%), GI.b (N=1, 1%) and GII.e (n=1, 1%) (Figure 12). Altogether, 108/112 (96%) of the NoV AGE cases were genogroup GII.
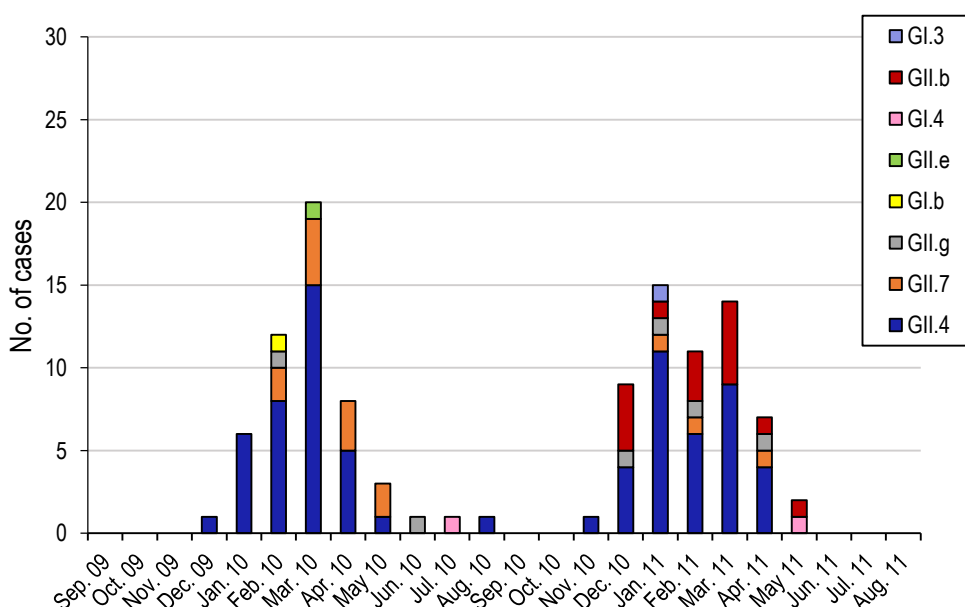
Figure 12. Norovirus genotypes seen in children between September 2009 and August 2011.

5.3 Severity of gastroenteritis caused by norovirus, rotavirus and adenovirus compared with gastroenteritis episodes of unknown etiology

The severity of the AGE cases with a NoV or RV as a single causative agent (and all the data was available for counting the score) were evaluated on a 20-point scale and compared with each other. Severity could be assessed in 84/180 (47%) of the NoV positive AGE cases and in 202/375 (54%) of the RV positive AGE cases with no other viruses found in stools. Besides these, severity was assessed in nine AdV positive cases with AdV as the only virus found and in 87 AGE cases in which all the stool tests for AGE viruses were negative (UNK).

Figure 13 shows the distribution of severity of NoV, RV and AdV AGE episodes assessed on the 20-point scale. The severity score of NoV AGE varied from 8 to 19, with a median severity of 14. The severity of RV AGE ranged from 10 to 20, with a median of 16. The severity of AdV AGE ranged from 9 to 16, the median being 14. The severity of the AGE cases with unknown etiology ranged from 5 to 19, with a median of 12.
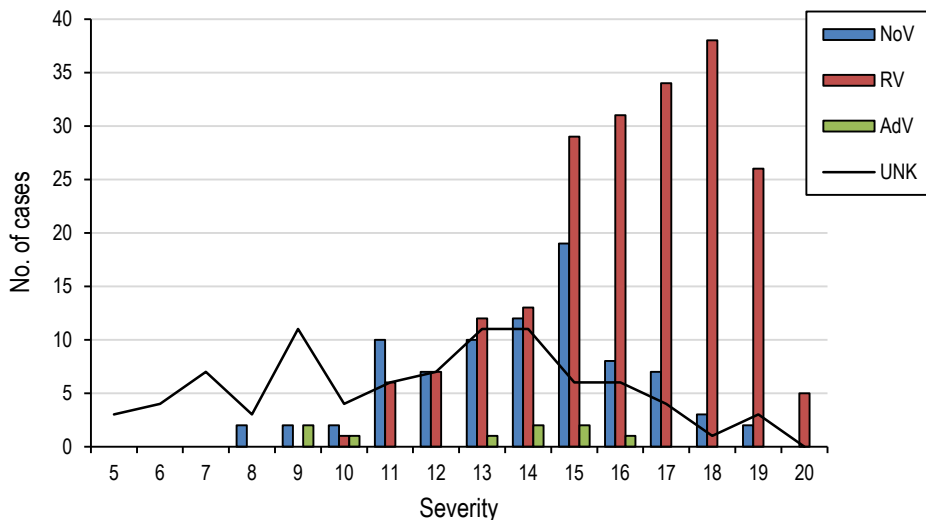
Figure 13. Severity of gastroenteritis cases on the 20-point severity scale, the Vesikari scale (34). NoV: norovirus; RV: rotavirus; AdV: adenovirus; UNK: gastroenteritis cases with all tested viruses negative.

The overall severity of RV AGE cases was higher than that of NoV AGE cases; however, the seasonal NoV AGE seen at the hospital was also remarkably severe. Severe cases of both NoV and RV AGE were seen at all ages.Separate AGE symptoms caused by RV, NoV or an unknown etiology (UNK) are shown in Table 3, in which the mixed AGE cases (with more than one virus) are excluded from the RV and NoV groups. Comparing the frequency of symptoms by causative agent, a higher fever was more common in RV AGE than in NoV AGE ($p<0.001$) or UNK AGE ($p<0.001$). High fever was also more common in NoV AGE than in UNK AGE ($p<0.001$). The degree of dehydration was significantly higher in RV AGE ($p<0.001$) and NoV AGE ($p<0.001$) than in UNK AGE, but there was no significant difference in dehydration between RV and NoV AGE ($p=0.345$). The duration of diarrhea was longer in RV AGE than in UNK AGE ($p<0.001$), but the difference between RV and NoV AGE ($p=0.113$) or between NoV and UNK AGE ($p=0.009$) was statistically non-significant. The duration of vomiting was significantly longer in both RV ($p<0.001$) and NoV ($p<0.001$) than in UNK AGE, as was the maximal number of vomits/day ($p<0.001$ between RV and UNK, and NoV and UNK in both). There was no statistical difference in the maximal number of diarrheal stools/day between the AGE episodes of other etiology. The variation and statistical significance of the individual symptoms by etiologic agents is described by incidence in Tables 3 and 4.

Characteristics		RV (N=202)		NoV (N=84)		UNK (N=87)	
		N	(%)	N	%	N	%
Diarrhea	Yes	201	(99.5)	83	(98.8)	79	(90.8)
	No	1	(0.5)	1	(1.2)	8	(9.2)
Number of days of diarrhea	1-4 days	24	(11.9)	21	(25.0)	26	(29.9)
	5 days	29	(14.4)	9	(10.7)	10	(11.5)
	$\geq$6 days	148	(73.3)	53	(63.1)	43	(49.4)
Maximum number of looser than normal stools/day	1-3	23	(11.4)	19	(22.6)	12	(13.8)
	4-5	32	(15.8)	17	(20.2)	17	(19.5)
	$\geq$6	146	(72.3)	47	(86.0)	50	(57.5)
Fever	Yes	187	(92.6)	37	(44.0)	56	(64.4)
	No	15	(7.4)	47	(56.0)	31	(35.6)
Maximum fever reported/day	37.1-38.4 °C	34	(16.8)	21	(25.0)	10	(11.5)
	38.5-38.9 °C	58	(28.7)	6	(7.1)	7	(8.0)
	$\geq$39 °C	95	(47.0)	10	(11.9)	39	(44.8)
Vomiting	Yes	200	(99.0)	81	(96.4)	57	(65.5)
	No	2	(1.0)	3	(3.6)	30	(34.5)
Number of days of vomiting	1 day	23	(11.4)	9	(10.7)	9	(10.3)
	2 days	29	(14.4)	11	(13.1)	15	(17.2)
	$\geq$3 days	148	(73.3)	61	(72.6)	33	(37.9)
Maximum number of episodes of vomiting per day	1	12	(5.9)	3	(3.6)	6	(6.9)
	2-4	45	(22.3)	24	(28.6)	29	(33.3)
	$\geq$5	143	(70.8)	54	(64.3)	22	(25.3)
Degree of dehydration based on clinical evaluation	No dehydration	7	(3.5)	5	(6.0)	29	(33.3)
	Mild/Moderate (1-5%)	168	(83.2)	70	(83.3)	56	(64.4)
	Severe ($\geq$6%)	27	(13.4)	9	(10.7)	2	(2.3)
Treatment	None	0	(0.0)	0	(0.0)	0	(0.0)
	Rehydration	94	(46.5)	47	(56.0)	45	(51.7)
	Hospitalization	108	(53.5)	37	(44.0)	42	(48.3)

Table 3. Symptoms of acute gastroenteritis episodes by different causative agents. RV: rotavirus; NoV: norovirus; UNK: gastroenteritis cases with all tested viruses negative

Factor of severity score and etiological comparison		p-value
Maximal number of diarrhea /day		
	RV $\geq$ NoV	0.013
	RV $\geq$ UNK	0.006
	NoV vs. UNK	0.582
Duration of diarrhea		
	RV vs. NoV	0.113
	RV > UNK	<0.001
	NoV $\geq$ UNK	0.009
Maximal number of vomites /day		
	RV vs. NoV	0.068
	RV > UNK	<0.001
	NoV > UNK	<0.001
Duration of vomiting		
	RV vs. NoV	0.345
	RV > UNK	<0.001
	NoV > UNK	<0.001
Fever		
	RV > NoV	<0.001
	RV $\geq$ UNK	0.008
	NoV > UNK	<0.001
Degree of dehydration		
	RV vs. NoV	0.345
	RV > UNK	<0.001
	NoV > UNK	<0.001
Total score		
	RV > NoV	<0.001
	RV > UNK	<0.001
	NoV > UNK	<0.001

Table 4. Comparison of statistical significance of gastroenteritis symptoms and total scores by etiology.

5.4 Assessment of the burden of rotavirus and norovirus gastroenteritis and associated costs

5.4.1 Burden of norovirus and rotavirus at the hospital level and rate of hospitalizations

Based on hospital records, AGE was the diagnosis for treatment in a total of 1723 children seen in the hospital in the two-year study period, out of which 534 children were hospitalized. We recruited 1193 (69% of the total) children for the study: 678 children treated as outpatients, 467 children who were hospitalized (87% of all hospitalized cases), and 48 children who caught a nosocomial AGE while hospitalized for another reason. Out of the enrolled children, 809 (68%) had adequate stool samples for viral studies. In the group with a stool specimen, there were more hospitalized children (49.8%) than in the total group (39.1%). Otherwise, there were no significant differences between the groups: the age range and gender were similar (Table 5).

Of all the 403 etiologically investigated hospital admissions due to AGE, 226 (56.1%) were caused by RV and 101 (25.1%) by NoV. Assuming the same proportional ratio is true for all the hospitalized cases in the area, the total number of RV hospitalizations in the two-year period was 300 (150 episodes/year) and of NoV hospitalizations 134 (67 episodes/year). In the hospital catchment area with a population of 85 000 children, the annual rate of RV hospitalizations in the study period was 17.6/10 000 children and of NoV hospitalizations 7.9/10 000 children 0-15 years of age.

Extrapolated from the Pirkanmaa District to the child population of whole Finland (8.9% of the children living in the catchment area), RV causes about 1690 and NoV 750 hospitalizations annually in children in Finland. Evaluating the number of RV hospitalizations based our study using the data from the second season (2007–2008) only, because of the atypically low RV occurrence in the first season, results in higher figures. Between September 2007 and August 2008, there was a total of 334 hospitalizations due to AGE in children <16 years of age at the study hospital (data from the hospital records), 66% (220 hospitalizations) resulting from RV infections. Extrapolating this to the whole of Finland, RVs annually cause 2480 hospitalizations of children less than 16 years of age.

		Studied for NoV and RV (N=809)		Total cohort (N=1193)	
		N	(%)	N	(%)
Sex	Female	358	(44.3)	541	(45.3)
	Male	451	(55.7)	652	(54.7)
Age	<6 months	78	(9.6)	93	(7.8)
	6-11 months	178	(22.0)	230	(19.3)
	12-17 months	144	(17.8)	206	(17.3)
	18-23 months	110	(13.6)	163	(13.7)
	2 years	122	(15.1)	188	(15.8)
	3 years	57	(7.0)	105	(8.8)
	4 years	34	(4.2)	59	(4.9)
	5-6 years	41	(5.1)	80	(6.7)
	7-8 years	25	(3.1)	39	(3.3)
	9-10 years	10	(1.2)	11	(0.9)
	11-15 years	10	(1.2)	19	(1.6)
Treatment	Hospitalized	403	(49.8)	467	(39.1)
	Outpatients	365	(45.1)	678	(56.8)
	Nosocomial	41	(5.1)	48	(4.0)
Total		809	(100.0)	1193	(100.0)

Table 5. Demographics of the children with stool samples (n=809), and all enrolled (n=1193) children.

Of the 809 cases with stool samples, 365 (45.1%) were treated as outpatients, 403 (49.8%) were hospitalized, and 41 (5.1%) were nosocomially acquired (Figure 4). RV was found in 226/403 (56.1%) and NoV in 101/403 (25.1%) of the hospitalized cases. Of the 365 cases treated as outpatients, 179 (49.0%) were positive for RV and 97 (26.6%) for NoV. Of the 41 nosocomial cases, 16 (39.0%) were RV-positive, and 15 (36.6%) were NoV-positive.

The duration of hospitalization varied from one to 16 days, with the mean of 2.25 days for NoVs and 2.48 for RVs. The median length of hospitalization was two days for both.

5.4.2 Costs of rotavirus and norovirus gastroenteritis

5.4.2.1 The medical costs: Hospitalizations and outpatient clinic visits

The daily bed charge, the amount charged from the community for a child hospitalized because of AGE, was €565/day. The cost of the hospital treatment charged to the community per episode ranged from €565 to €4178: with the median length of hospitalization of two days, the average cost of hospitalization charged to the community was €1130. Calculated from the average cost of hospitalization and the number of hospitalized RV and NoV AGE cases, the costs to communities for hospitalizations in the two-year period due to RV AGE was about €339 000 (€169 500/year) and those due to NoV AGE about €151 400 (€75 700/year) in the hospital district.

Apart from the hospitalized cases (n=534) and nosocomial cases (n=54), AGE cases (n=1135) were treated as outpatients. Of these, the community was charged €335/visit regardless of causative pathogen. Assuming that the ratio of RV vs. NoV (49.0% vs. 26.6%) AGE is the same in all the enrolled cases as it is in the microbiologically identified cases, about 560 of the cases were caused by RV and 300 by NoV. Thus the total cost of RV AGE in outpatients for the two-year period would total about €187 600 (€93 800/year) and of NoV AGE about €100 500 (€50 300/year) in the hospital district.

Extrapolated from the catchment area of the Tampere University Hospital to the whole of Finland, the annual numbers of RV hospitalizations and outpatient visits were about 1690 and 3120, and those of NoV hospitalizations and outpatient visits 750 and 1700, respectively. Estimated from these figures, the annual medical care costs for RV AGE total €2 950 300 and for NoV AGE €1 419 400 at the hospital level in Finland (Table 6).

	Rotavirus	Norovirus	NoV/RV ratio
Hospitalized:			
Number of episodes	1685	753	45%
Unit cost, hospital treatment	€1130	€1130	-
Total, hospital treatments	€1 904 100	€850 900	45%
Outpatient clinic visits:			
Number of episodes	3123	1697	54%
Unit cost, outpatient clinic treatment	€335	€335	-
Total, outpatient clinic treatments	€1 046 200	€568 500	54%
Total costs:	€2 950 300	€1 419 400	48%

Table 6. Annual medical costs of rotavirus and norovirus gastroenteritis in children seen at the hospital, estimated for whole of Finland.

5.4.2.2 Lost working days

The results concerning lost working days were estimated based on the information obtained from interviewing 243 families with children hospitalized because of AGE. Missed working days by family members or another person caring for the child were reported in 149 of 243 (61.3%) AGE episodes. The median number of workdays lost was two for both RV and NoV AGE. The social security benefits for one missed working day in 2007, based on the average salary in Finland in 2007, was €75. Extrapolated to all AGE episodes, an average cost of €92 per AGE episode was incurred by society (as missed workdays were reported in 61% of the cases, and when they occurred the mean cost for society was €150/episode).

5.4.2.3 Costs for the families

The direct, "out-of-pocket" cost to families was calculated based on information acquired from interviewing 243 families with children hospitalized because of AGE (Table 7). The reason for choosing medians in estimating the cost was, that using

the mean costs single exceptionally high expense values (listed as "maximums" in Table 7) would have possibly overestimated the results.

The cost of hospital treatment paid by parents varied from €26 to about €600, with a median of €74; this includes also the parents' expenses while staying with the child. The major part of the other out-of-pocket costs was transportation: travelling the distance between home and hospital by car or public transportation. The maximum cost of using one's own car was €87 and public transportation or taxi €552, with respective median costs of €9.50 and €40. The maximum cost for medication and oral rehydration solution (ORS) for AGE treatment was €30 and €57 respectively, with medians of €4.75 and €11.90. The other out-of-pocket costs, for example extra diapers or laundry expenses, ranged from €0.40 to €275, with a median of €17.50.

Altogether, the average out-of-pocket cost for the families of hospitalized children was €115 per one AGE episode. Table 7 summarizes the categories, means, medians and maximum amounts of the costs paid by family.

The total indirect costs, the out-of-pocket expenses and social benefits totaled an average of €207 for hospitalized cases and €133 for the cases treated as outpatients.

		RV+ (N=151)		NoV+ (N=83)		All cause (N=243)	
		Value or n	%	Value or n	%	Value or n	%
Need for medication	Yes	72	47.7	38	45.8	105	43.2
	No	79	52.3	45	54.2	138	56.8
Cost of medication (€)	Mean	10.09		11.42		10.02	
	Minimum	3.62		3.78		2.50	
	Median	4.38		5.00		4.75	
	Maximum	26.66		30.00		30.00	
Need for ORS	Yes	108	71.5	59	71.1	158	65.0
	No	43	28.5	24	28.9	85	35.0
Cost of ORS (€)	Mean	18.08		18.46		18.00	
	Minimum	0.00		5.80		5.80	
	Median	14.00		15.00		11.90	
	Maximum	57.00		45.00		57.00	
Public transportation or taxi	Yes	36	23.8	23	27.7	60	24.7
	No	105	69.5	57	68.7	171	70.4
	Unknown	10	6.6	3	3.6	12	4.9
Cost for public transportation	Mean	49.31		93.48		67.29	
	Minimum	2.00		9.20		2.00	
	Median	36.45		54.00		40.00	
	Maximum	180.00		552.60		552.60	
Use of own car	Yes	136	90.1	73	88.00	219	90.1
	No	5	3.3	7	8.4	12	4.9
	Unknown	10	6.6	3	3.6	12	4.9
Cost of using own car (€)	Mean	14.80		15.50		14.40	
	Minimum	0.40		0.40		0.40	
	Median	9.50		11.90		9.50	
	Maximum	87.20		73.00		95.20	
Other costs (e.g. laundry, diapers)	Yes	136	90.1	77	92.8	222	91.4
	No	0	0.0	0	0.0	0	0.0
	Unknown	15	9.9	6	7.2	21	8.6
Other costs (€)	Mean	26.20		37.26		29.17	
	Minimum	0.40		0.40		0.40	
	Median	15.85		22.50		17.50	
	Maximum	275.00		184.00		275.00	

Table 7. Out-of-pocket costs resulting from an AGE episode in a child in a family. ORS: oral rehydration solution.

5.4.2.4   Total cost of rotavirus and norovirus gastroenteritis episodes

To assess the total cost of NoV and RV AGE in children to the families and to society, we added together the cost of medical care (the cost of AGE hospitalization and outpatient clinic treatment), the costs to society of missed working days, and the out-of-pocket costs for the families. The total annual cost was about €3 714 500 for RV AGE and €1 801 000 for NoV AGE. The cost of NoV AGE was 48% of that of RV AGE (Table 8).

		Norovirus	Rotavirus	Ratio of NoV/RV
Hospitalized:				
Number		753	1685	
Unit cost, hospital treatments		€1130	€1130	
Total, hospital treatments		€850 900	€1 904 100	45%
Unit cost, indirect expenses		€207	€207	
Total, indirect expenses		€155 900	€348 800	45%
	Total	€1 006 800	€2 252 900	45%
Outpatient clinic visits:				
Number		1697	3123	
Unit cost, outpatient clinic treatment		€335	€335	
Total, outpatient clinic treatments		€568 500	€1 046 200	54%
Unit cost, indirect expenses		€133	€133	
Total, indirect expenses		€225 700	€415 360	54%
	Total	€794 200	€1 461 560	54%
Total costs:		€1 801 000	€3 714 460	48%

Table 8.   Annual numbers and estimated medical and indirect costs of acute gastroenteritis episodes caused by norovirus and rotavirus in children at hospital level in Finland.

5.5   Other causative agents of gastroenteritis (this study)

5.5.1   Adenovirus

Enteric AdVs were found in 25/809 (3%) AGE cases during the two follow-up seasons: 9/341 (3%) cases in the first season and 16/468 (3%) in the second. If the two cases associated with the Pirkanmaa waterborne AGE outbreak are excluded from the second-season material, there were 14 of 418 (3%) AdV AGE cases

found then and a total of 23 cases of 759 (3%) in the two follow-up years. Among the cases with enteric AdV detected from the stools, there were six cases in which also RV (five G1P[8] and one G2P[4]) was found, one case in which also NoV GI.3 was found, and one case in which also SaV was found: the latter two cases were associated with the Pirkanmaa waterborne AGE outbreak. Altogether, enteric AdV was the only AGE pathogen found in 17 cases.

Of the AdV AGE cases, one child was less than six months of age, eleven were 6-23 months, seven were 2-3 years, and six were 3-5 years of age.

The severity of AdV AGE could be assessed in nine cases with AdV as the only virus found in the stools. Among these cases, there were no mild cases (1-6 points), three cases were of moderate severity (7-10 points), and six cases were severe (≥11 points) according to the 20-point severity score (Figure 13).

5.5.2 Sapovirus

SaVs were found in 23/809 (3%) of the AGE cases: three cases (of 341 AGE cases, or <1%) in the first AGE season; and 20 (of 468, or 4%) in the second. Of the latter, 11/20 were associated with the Pirkanmaa waterborne AGE outbreak. Excluding these cases, nine seasonal SaV AGE cases (of 418, or 2%) were found in the second season and 12 seasonal SaV AGE cases (of 759, or 2%) during the two follow-up years.

The severity could be assessed in 12 of all the SaV positive AGE cases. Of these cases, 11 (92%) were classified as severe. However, six of the severe cases were associated with the Pirkanmaa outbreak, and five of them were mixed infections with other viruses also detected in the stools.

Disregarding the SaV positive cases associated with the Pirkanmaa outbreak, all the remaining 12 cases were seen in children <3 years of age; more precisely, six of these children were less than 18 months of age. Two of the cases were also positive for RV. Severity could be assessed in five of the 12 cases, whose severity scores were 10, 12, 13, 15 and 18, the last-mentioned one of the mixed infections with SaV and RV.

5.5.3 Aichi virus

AiVs were found in 28 cases: one case (of 341, or <1%) in the first season and 27 (of 468, or 6%) in the second. However, 25 of the 27 AGE cases seen in the

second follow-up season were associated with the Pirkanmaa outbreak. Excluding them, there were two AiV AGE cases (of 418, or <1%) found in the second season and three (of 759, or <1%) in both seasons counted together. In one of these three AiV positive AGE cases, there was also RV (G1P[8]) and in one case NoV (GII.4) present in stools, leaving one AiV positive case in which no other enteropathogenic viruses were found. This child was four months old and treated in the outpatient clinic. Interestingly, this case was not reported to be associated to the Pirkanmaa outbreak, but it did appear at the same time as the peak of the outbreak.

5.5.4 Bocavirus

HBoVs were found in 77 AGE cases: 34 cases (of 341, or 10%) in the first season and 43 (of 468, or 10%) in the second. Excluding the cases (n=10) associated with the Pirkanmaa outbreak, there were 33 AGE cases with HBoV (of 418, or 8%) found in stools in the second season and 67 (of 759, or 9%) cases in both seasons together.

Among the 77 cases of AGE and HBoV found in the stools, there was at least one other AGE-causing agent found in the stools in 71/77 (92%) children. Furthermore, 38/77 (49%) had also respiratory tract infection symptoms. In fact, there was only one AGE case in which no viruses other than HBoV were found in the stools, and with no symptoms or signs of respiratory tract infection. The more detailed results of the HBoV studies have been published elsewhere (262).

5.5.5 Coronavirus

HCoVs were found in 19 AGE cases: in eight cases (of 341, or 2%) in the first season, and 11 (of 468, 2%; or 11/418, 3% excluding the cases associated to Pirkanmaa outbreak) in the second season. There were no HCoV positive cases associated with the Pirkanmaa waterborne AGE outbreak. However, HCoVs were usually seen together with one or more AGE-causing viruses, or in children who also had symptoms of respiratory infection. There was only one case in which there were no other viruses or respiratory symptoms found. The HCoVs were considered to be insignificant viruses in terms of AGE etiology: in other words, as not-AGE-causing viruses (261).

5.5.6   Other

In the study material, there were 162/809 (20%) AGE cases in which stool specimens had been obtained for analysis, but all the virus tests were negative. Among these, there were nine *Salmonella* cases, five *Campylobacter* cases, and two *Yersinia* cases: altogether 16 cases with a bacterial etiology for AGE. Also found in stools were *Clostridium difficile* in two cases, EHEC in one case, and *Blastocystis Hominis* in one case. In one case, the diagnosis was hemolytic-uremic syndrome with an unknown etiology.

In 51 cases with an unknown etiology for the AGE symptoms, there were also respiratory tract infection symptoms present. In eight cases, there was a diagnosis other than AGE after admission to hospital and enrollment in the study: two cases of Kawasaki disease, two cases with abdominal abscess, one case of Henoch-Schönlein purpura, one pineoblastoma, one Hodgkin's disease, and one pyloric stenosis.

5.6   The Pirkanmaa waterborne gastroenteritis outbreak (I)

A total of 115 children $\leq$ 15 years of age were seen at the Tampere University hospital because of AGE associated with the Pirkanmaa outbreak in November-December 2007. Of these, 65 children were enrolled in this study, and stool samples were obtained from 50 cases. All the stool samples were tested for RVs, HuCVs, AdVs, HBoVs, HCoVs and AiVs using PCR, and the positive amplicons were sequenced to confirm the result and determine the genotype of the virus. Besides viruses, the enteropathogenic bacteria had been studied for clinical purposes in 33 (out of 50) cases in the hospital microbiology laboratory.

Of the 65 enrolled cases, 37 (57%) were treated as outpatients, and 28 (43%) were hospitalized. The majority of the stool samples (28/50, or 56%) were obtained from the hospitalized cases, whereas some of the enrolled children were discharged from the outpatient clinic before stool samples had been obtained. The severity of the cases could be assessed in the hospitalized children only, because the clinical information collected from the children treated as outpatients was insufficient for assessing the severity score.

5.6.1   Causative agents of gastroenteritis in the outbreak

The majority of the cases (33/50, or 66%) had different combinations of mixed AGE with more than one causative agent. In three cases (6%) no causative agent could be found, and in seven cases (14%) only one virus was found in the stools. There were no cases in which only bacterial agents were found.

The combinations of viruses found in the stools are listed in Table 2 in the original Study **I.** The major causative agents of the AGE in children in this outbreak were RV and NoV: RV was found in 33/50 (66%) cases and NoV in 20/50 (40%) cases. Also AiV was present surprisingly often, in 25/50 (50%) cases, but it was mainly seen in combinations with other causative agents. Only in one case was AiV the sole enteropathogenic agent found, but in this case no bacterial culture had been cultivated. The maximum number of causative agents found in a single case was seven: five different viruses (NoV, RV, AdV, AiV and HBoV) and two enteropathogenic bacteria (*Salmonella* species, *Campylobacter jejuni*). A large variation among other viral enteropathogen combinations was seen, but there were no cases in which more than one type of HuCVs were found.

5.6.2   Severity of the gastroenteritis cases in the outbreak

The AGE cases seen in children in this outbreak were severe. The severity was assessed in 28 cases, of which all the data needed for assessment of the severity and a stool sample were available.

On the 20-point scale, the median severity of these AGE cases was 17. The severity was not dependent on the causative agent(s): equally severe cases were seen in cases caused by single virus, combinations of viruses, and combinations of viruses and bacteria. This probably reflects the massive infective doses of the enteropathogens that the affected persons were exposed to. The only remarkable difference between the symptoms appearing with the different causative agent(s) of AGE was that bloody diarrhea was seen only in the AGE cases in which campylobacter was present.

A closer look at individual symptoms of AGE revealed that both diarrhea and vomiting were frequent and long-lasting. Of the 28 cases, the maximum number of diarrheal stools was $\geq$10/day in 16 cases (57%), and the diarrhea lasted for a week or more in 20 cases (71%). The maximum amount of vomiting was $\geq$10 times/day in ten cases (36%), and vomiting lasted for a week or more in six cases (21%). A fever of >38ºC was seen in 20 cases (71%), and in nine cases (32%) it was $\geq$39ºC.

6   Discussion

6.1   Medical and financial burden of rotavirus gastroenteritis in children

In this thesis the significance of RVs in AGE in children at present was assessed and summarized since RVs were identified in the 1970s. The study was planned as the last opportunity for a prospective follow-up study on incidence, causative viruses, and the medical and financial burden of AGE in Finnish children before implementation of the universal RV vaccination. A prospective hospital-based study of AGE in children was conducted in Tampere in 1977-1978 (10), but there have been no other comparable studies in between. For RV, we wanted to use RT-PCR for primary detection in addition to standard ELISA, assuming that more cases could be detected by RT-PCR than by ELISA (112). We also wanted to determine the RV genotypes, which could not be studied in the previous study in Tampere. Genotypes have been determined, however, in prospective surveillance of participants in RV vaccine studies, for example in 1993-1995 (117), and the studies of RV5 and RV1 in the 2000s (264,265,325).

The first finding was that the epidemiology of RV AGE had not changed from 1977–1978 to the present study.

In the study by Vesikari and Mäki et al. (8), 280 stool samples of children seen at the Tampere University Hospital because of AGE were examined by radioimmunoassay or EM. RV was found in 49% of the cases and AdV in 11%; the other cases were considered as "non-RV, non-AdV" cases. This finding is in line with our findings with 38% of AGE cases being RV positive in the first season and 63% in the second. Also the age distribution of children seen because of RV AGE was similar to that in our study, with the majority of the patients 7-18 months of age.

In this study, we used both ELISA and RT-PCR in detecting RVs from stool specimens. In a former study, Pang et al. (112) found that PCR detects more cases than ELISA, but the false negative cases by ELISA were of mild severity and presented proportionally more of the uncommon RV strains. In our study, the sensitivity of ELISA method was good, finding 91% of the RT-PCR positive RV

cases and no false positives. In the severe AGE cases in the hospital-based material, ELISA worked well; in fact, the PCR methods may be even oversensitive and find RV shedding in cases where the causative agent of AGE symptoms is another virus. Our results prove that ELISA still works fine in detecting RV AGE cases in clinical practice and in epidemiological studies, when the genotype does not need to be determined. In this study the genotype distribution of the PCR+/ELISA- cases was similar to ELISA+ cases.

A clear seasonality was seen in RV AGE. The first season, which featured an unexpectedly low RV activity overall, had a late beginning and it lasted until the summer months: June, even into July. The second season was a more typical RV season altogether, having begun in the autumn months of October–November. Also this season lasted until the summer. These suggest that the timing of the RV seasons might really be getting delayed and moved towards the summer. The inactive months in RV epidemiology were in the late summer and early autumn.

An estimate of the burden of RV AGE in Finland, based on data on hospital admissions between 1985 and 1995, concluded that 1935 0-2 year old children, or 3% of all Finnish children in that age group, are hospitalized annually because of RV AGE (61). Furthermore, the study group evaluated that RV annually caused about 2900 outpatient clinic visits at the hospital, 8320 medical consultations, and 5030 AGE cases treated at home in Finnish children. The epidemic RV season lasted from November or December until June or July. The predominant RV genotype was G1 except for the 1988–89 season, in which G4 was prominent (61).

In a more recent report by THL in 2007 for purposes of evaluating the utility of RV vaccination in the NIP in Finland, the annual rate of RV hospital admissions was estimated to be 2400, hospital outpatient visits 3600, and primary care consultations 9000 for Finnish children (118). In addition, RV was estimated to cause 0.5 deaths annually (118). These figures are similar to the results of our study if the RV burden is evaluated based only on the 2007–2008 season. The incidence figures for the 2006–2007 RV AGE season were so low that they make our figures for the RV burden too small, if the average of the two seasons is used.

When this study was designed, the above-mentioned evaluation by THL had not yet been made and, in any case, a prospective study can reflect the reality better than reports based on extrapolation. At the launch of this study, two RV vaccines had just been accepted for clinical use in Finland, and they were available at people's own expense. It was not known whether or when the RV vaccines would be adopted as part of the Finnish national vaccination program. The vaccines were known to be efficacious, but there was debate about whether replacement of

prevented RV AGE by other RV genotypes or replacement of the prevented RVs by other AGE viruses could become an issue.

The severity of AGE episodes caused by different viruses was compared to each other using the 20-point severity score. We analyzed only the severity scores of those AGE cases in which all the needed information was available either from the hospital records or from interviewing the parents. Certainly, the fact that all the cases were severe enough to indicate referral to hospital for pediatric consultation affects the overall perception of their severity. The scores are at the high end of the scale, indicating severe AGE cases. However, even if the cases for which scores were determined do not represent the entire scale of RV and NoV AGE, the results provide us with a good idea of how severe episodes RVs and NoVs can cause and that such severe cases are not rarities.

The overall severity of RV AGE was higher than AGE episodes with other etiologies. In RV AGE, fever was more often present compared to NoV AGE (in 92.6% vs. 44.0%), and the fever was at least 38.5°C in more than 75% of the cases. Also the duration of diarrhea and the maximal number of diarrheal stools per day seems higher in RV than in NoV AGE, even if these differences did not reach statistical significance. These results confirm the notion about RVs being the causative agent of the most severe forms of childhood gastroenteritis. These results also show the relative difference between the severity of RV and of NoV AGE cases, RV AGE being more severe than the others are, on average.

The two consecutive AGE follow-up seasons were quite different from each other. In the years before RV vaccinations, when AGE was a very common cause of hospitalization of children, it was common knowledge that the RV seasons were heavier in some years than others. This phenomenon was shown in this study as well. RV activity was unexpectedly low in the first follow-up season, and a substantial share of the RV cases seen at the hospital was caused by genotypes that had been rare in earlier studies in Finland (61,326). We assume that the beginning of vaccination against RV may have interfered with the "normal" transmission of RVs in young children, causing a low season and uncommon circulating strains. The same kind of phenomenon was reported in the US in 2008, with a RV vaccination coverage of 46%. The hospitalizations there due to RV AGE decreased in the first year in both vaccine-eligible age groups and in older age groups. In the latter group, the indirect beneficial effect disappeared in 2009, suggesting interference in RV epidemiology by vaccination (294).

In the second follow-up season, a return to the expected RV epidemiology was seen. The number of hospitalizations due to AGE was much higher than in the

first year, and RVs were clearly the major causative agents of AGE in children who were seen at the hospital level.

In Finland, the most common RV G-type in children in earlier studies was G1, followed by G4, G2 and G3 (61,326). In Western countries, the five common RV genotypes found in etiologic studies in the 2000s are G1P[8], G9P[8], G2P[4], G3P[8] and G4P[8] (77,84).

Keeping in mind the debate about the possible role of RV vaccinations in shifting the circulating RV genotypes towards the non-vaccine types, the relatively large proportion of G2P[4] was especially interesting. Despite the rising RV vaccination rate, the second follow-up year was a return to the common frequent genotypes, suggesting that the occurrence of rare genotypes was natural year-to-year variation rather than vaccine-induced genotype replacement or, if vaccine-induced it was only temporary. Aside from the above-mentioned five major RV genotypes, no other RV types were seen in this study.

In further follow-ups on circulating RV genotypes after RV vaccination was introduced to the NIP in Finland, no surprises indicating genotype replacement have appeared. The predominating RV strains seen among the decreasing amount of RV AGE cases in 2009–2011 were G1P[8] and G4P[8]. Also G9P[8] and G3P[8] were found in the post-vaccination years (Study **IV**).

It is difficult to obtain and evaluate the costs caused by an AGE episode for society and for families, and to extrapolate the results to larger populations. Treatment practices are different at different hospitals, and this affects how much communities are charged: at the Tampere University Hospital, for example, it is common practice for the rehydration therapy in AGE treatment to be performed per orally (often via nasogastric tube) in the outpatient clinic, and hospitalization is quite rarely needed.

The total direct cost of AGE caused by NoV or RV would be the sum of costs of hospitalizations, outpatient clinic visits and physician (office) visits. The estimates of the cost of hospitalizations and clinic visits presented here are based on actual data collected in a large hospital district, whereas we did not collect data on primary care visits. In an evaluation by the National Public Health Institute of Finland (THL) of the economic impact of RV vaccines in Finland based on the years 1999–2004 prior to universal RV vaccination, there were 9000 primary care visits from children less than five years of age (118). For a total of 9000 physician visits at a year (€70 per visit) the direct medical costs for RV-related visits would be €630 000 per year for Finland.

The same THL analysis estimated the number of annual hospitalizations to be 2400 and outpatient clinic visits to be 3600. These are higher numbers than the extrapolations from our study. In our study, the proportion of NoV AGE hospitalizations to RV AGE was about 45% and outpatient clinic visits about 54%, whereas comparison with the THL analysis would yield a lower figure. The timing of our study may explain the difference: in 2006–2008, the RV vaccine coverage in the private market had already increased from 9% to 35%, and the vaccinations had probably reduced the number of RV hospitalizations during the study period. Particularly the first RV season in our study, 2006 to 2007, showed a very low level of RV activity.

The medical costs of RV AGE cases seen at the hospital were €2 950 300. Adding the same costs for primary care visits mentioned above, €630 000, the total medical costs for RV would be €3 580 300. Our results support the THL analysis of benefits of including the RV vaccine in the NIP of Finland.

6.2   Medical and financial burden of norovirus gastroenteritis in children

This study confirmed that the burden of NoV AGE in children at the hospital level is significant. NoVs were the cause of a total of 23% of the AGE cases seen at the Tampere University Hospital during the two follow-up years: as common as RVs, or second most common after RVs as the causative agent of AGE. Almost half (47%) of the NoV AGE cases seen required hospitalization. Based on the study period, NoVs cause about 70 hospitalizations per year at the hospital, or 785 annual child hospitalizations extrapolated to all of Finland.

It was recently estimated that NoVs cause 102 deaths annually in children <5 years of age in Europe, or, in other words, 1/51 111 of the NoV AGE episodes in Europe are lethal (227). NoVs have been assessed to cause 12% of all severe AGE cases in children, with an incidence of 12 hospitalizations per 10 000 children, and 167 medical consultations per 10 000 children (144). In Finland, this would mean 1150 hospitalizations and 15 700 medical consultations of children annually because of AGE.

As discussed in Chapter 6.1.1. above, in this study the severity of analyzed cases is deviated towards more severe, because the study is hospital-based, and because the clinical records were more commonly inadequate for the patients seen in the outpatient clinic. However, the difference in severity between RV AGE and NoV

AGE is apparent. Assessed using the 20-point severity score, in which an AGE episode with a score of ≥11 points is considered severe, also NoV AGE cases scored very high, with median of 14. Even if the material does not represent average AGE cases in children and the majority of the cases are milder and treated in primary care, it is noteworthy that NoVs do frequently cause severe AGE. Comparing single symptoms or signs of AGE, the symptoms in NoV AGE cases rated almost as severe as in RV AGE cases.

Comparing NoV AGE cases (n=84) to the cases with all the virus tests negative (n=87), the overall severity of NoV age was significantly higher (p<0.001). Also the number of vomiting times, the duration of vomiting in days, the level of fever and degree of dehydration were significantly higher in NoV AGE than in AGE with unknown etiology. This finding backs up the notion of NoV AGE as "a vomiting disease," even if there were no differences between NoV and RV in these symptoms.

The activity of NoV AGE showed a clear seasonality similar to that of RVs. The NoV and RV seasons overlapped but were not the same: they started and ended independently of each other. In addition, the first NoV season, which was a low RV season, showed high level of NoV activity; and the second, which was normal in terms of RV activity, was a quite active NoV season as well. In between the seasons was a six-month period with almost zero NoV activity.

The predominance of GII.4 over other NoV types in seasonal AGE was evident in both of the follow-up seasons in our study: other NoV genotypes were rare. This is probably the real epidemiologic situation in Finland. Especially in the first follow-up season, the NoVs other than GII.4 were single findings, even if the number of NoVs detections was higher than in the second season. In sequence analysis of the NoV GII.4., it was found that two new NoV GII.4 variants – 2006a and 2006b – emerged in the first season, i.e. 2006–2007. Of these, especially 2006b caused severe AGE cases needing hospitalization (327). In the second season, GIIb (or GII.3, as the genotype was later found to be) circulated alongside the GII.4 as a seasonal AGE causative agent.

As it was seen in the Pirkanmaa waterborne AGE outbreak, group GI NoVs were also in circulation, but among the seasonal AGE cases, the NoVs belonging to genogroup GI were not seen, except for single findings. This observation supports the concept that NoVs belonging to the genogroup GI are more commonly seen in foodborne or waterborne outbreaks (150,154,175-178), whereas group GII NoVs are usually seen in seasonal AGE in children and adults.

There are many uncertainty creating factors in estimating the total cost of NoV AGE in Finland. NoV is usually not tested for in primary health care and, moreover, large share of the NoV AGE episodes are mild enough to be treated at home without medical consultation. We know from prospective studies in the 1990s that the incidence of AGE in children under two years of age – including mild cases – attributable to NoV was actually somewhat higher than for RV (17,28). Therefore, we may conservatively assume that the number of primary care visits and the cost of NoV AGE would be about the same as those of RV AGEs: €630 000 per year.

We decided to use the median values instead if means in evaluating the medical costs and out-of-pocket costs of NoV and RV AGE. As a result, the estimated costs are probably lower than in reality. However, using the means with single very high costs and extrapolating from these to the whole of Finland, we might have ended up with unrealistically high costs: we decided to present conservative results to avoid exaggeration.

The medical costs for NoV AGE cases seen at the hospital were €1 419 400. Adding the cost of primary care visits as explained above, i.e. the same cost as estimated for RV AGE, €630 000, the total medical cost of NoV would be €2 049 400. The ratio of the total cost of NoV to that of RV is about 57%. Accordingly, the cutoff price of a future NoV vaccine with effectiveness similar to that of the current RV vaccines would be higher than that of the RV vaccine (or vaccination with a full series), i.e. about €50/child, which should still be affordable for many countries. The calculation would be more favorable if, instead of giving live oral RV vaccine and injectable NoV vaccine separately, RV vaccine was also replaced by a non-live RV vaccine. These two vaccines are combined in a currently proposed candidate vaccine (132,328).

Adding the indirect costs to the direct medical costs discussed above for an estimate of the total cost of AGE episodes necessitates some assumptions in the calculations. The interview forms we used in collecting information on out-of-pocket costs from the families had some shortcomings. Detailed information about these costs were collected, but even if we did obtain the information about the number of families with costs including defined categories, and the total of these costs, we did not obtain data on the combined costs to a single family. However, the out-of-pocket costs were very small compared to medical costs and other indirect costs to society, and they are not significant on the large scale.

The benefits of a NoV vaccine would not be restricted to children. As NoVs cause the majority of AGE episodes in all age groups, both children and adults,

preventing NoV AGE episodes would also decrease the number of lost working days, not only sick days. Vaccinating children would cut off a significant reservoir of NoV AGE, even if the circulation of NoVs as a whole would not be aborted. It is assumable that the herd effect would occur with NoVs to some extent: when the number of prone persons in a population decreases, the risk of infection is smaller also for the prone people. Besides children, also the elderly and people in risky professions, such as food employees, healthcare and childcare personnel, might be good targets for a vaccination.

6.3 Other causative agents of acute gastroenteritis in children

The known or suggested enteropathogenic viruses other than RV and NoV did not play a significant role in the epidemiology of seasonal AGE in children at the hospital level in this follow-up. Enteropathogenic AdVs were seen to the same extent as in earlier studies. SaVs can be potential causative agents of seasonal AGE or AGE outbreaks in children, but they seem to be much more uncommon than the NoVs are. In the two consecutive seasons of this study, SaVs were not epidemic viruses. It is interesting that also AiVs were found, but the role of these viruses in seasonal AGE in children seems to be marginal. HBoVs and HCoVs were mainly seen in cases with both respiratory and enteric symptoms. The share of the other AGE causing viruses might have been bigger if the cases seen at primary-care level and the cases not needing medical consultation were studied.

In 20% of the stool specimen, all the AGE viruses tested negative. However, looking back at the documents for these cases, there were also respiratory tract symptoms reported in 51/162 (31%) of these cases, and the infection might have been a respiratory tract infection with some symptoms from gastrointestinal tract.

There were 16 cases with a bacterial etiology of AGE, consisting of 2% of all the AGE cases with stool samples collected. If these cases were excluded from the analysis, the share of RV and NoV among AGE in children would be greater than the figures of our analysis.

6.4 The Pirkanmaa waterborne gastroenteritis outbreak

The Pirkanmaa AGE outbreak in 2007 was by far the largest published waterborne infectious epidemic (151). There are several unique features in this outbreak, e.g.

the variety in the multiple simultaneous enteropathogen combinations of co-infections and the extreme severity of many of the AGE cases without correlation of the severity with the identified pathogens.

Indeed, this outbreak can be seen as a massive experiment in what happens when a large amount of heavily fecal-contaminated water is made available for thousands of people to drink. Based on the results, it seems that, with such a massive viral load offered to children, the severity of the resultant AGE is high, regardless of the causative agent(s) or the age of the child. This finding is probably affected by the fact that the cases we were able to analyze presented the very extreme end of the AGE continuum: the children who were most seriously ill and hospitalized. It is possible that, among the cases treated as outpatients or at home, there would have been a correlation between the AGE causative agents and the severity of the illness.

On the other hand, it is obvious that multiple enteropathogenic agents contaminated the water that caused these AGE cases. Consequently, there were several infective and irritating agents affecting the children's gastrointestinal tract, regardless of which AGE causative agents were identified by laboratory tests.

Another thing that cannot be easily proven in "normal" AGE material was the finding that, despite the various enteropathogen combinations found, there were no cases in which more than one type of HuCV was found at the same time. There were several different NoV genotypes of both genogroups, GI and GII, found in the stool samples, indicating that numerous NoVs were available, but in no case was there more than one NoV type found at the same time. Furthermore, no co-infections with NoV and SaV were found. It suggests, that even with this kind of heavy inoculum, relative viruses act as antagonists to each other; or the stronger virus may reduce the capability of reproduction of the weaker one, and more than one HuCV cannot infect a person at the same time. It is also possible that the stronger virus disturbs the detection of relative viruses in RT-PCR.

One of the results of this outbreak analysis is that when a heavy dose of AGE causative agent has been drunk or eaten, the following AGE can be much more severe than an AGE caused by "usual" dose of infective agent, which enters the gastrointestinal tract through the mouth commonly via contaminated hands. This could also be one explanation to why common diarrheal diseases in developing countries cause child mortality at so much higher a level than in developed countries. Besides poor nutritional status and a lack of health services, the level of contamination of drinking water could also be a possible co-factor in mortality.

This outbreak also shows that numerous enteropathogenic agents do circulate among people without marked signs of an epidemic, and if there are favorable (or unfavorable) circumstances, very serious and variable infections can occur. The Pirkanmaa outbreak happened at the beginning of annual RV season. A few AGE cases caused by RVs and, to a lesser degree, by NoVs, had already been seen at the hospital. The RVs found in the stools of outbreak-associated cases were mostly G1P[8], which was the epidemic genotype in that year. There was much more variation in the NoV strains detected. Many other AGE causative agents were also found in lesser amounts in the sewage-contaminated water.

6.5   The impact of rotavirus vaccines

We expected that the relatively low RV level of vaccination coverage, rising from 6% to 35% during the follow-up period in 2006–2008, would not yet have a significant impact on the AGE epidemiology. However, it is possible that the unusually low RV activity in the first follow-up season (2006–2007) and high activity in the second (2007–2008) reflects the introduction of RV vaccines onto the market. Possibly even the low vaccination coverage in the target population could have diminished the number of RV-prone children in the first year, reducing the circulation of RVs, and when the size of the susceptible population grew moving towards the next year, the incidence of RV AGE was able to rise again. Such a phenomenon has been seen elsewhere (294).

In Finland, hospitalizations due to RV AGE decreased 76% comparing the two pre-NIP and two post-NIP years (**IV**). This follow-up has continued, and the impact has remained stable or improved further (see 5.1.6.). In the United States, a biennial pattern of RV seasons emerged after implementation of a RV vaccine, with lower and delayed seasonal peaks every other year (329,330). This phenomenon has been explained by an accumulation of susceptible children until RV epidemics can be transmitted efficiently in the US, where vaccination coverage has risen from 43.9% in 2009 to 72.6% in 2013 (331). This biennial pattern has not been seen in Finland, possibly because of a very high coverage of RV vaccination.

In the years before the launch of RV vaccines, there was a concern whether the vaccines with a high coverage would affect unfavorably the epidemiology of circulating RVs. The genotype replacement can be seen especially with such infective agents, which induce mostly homotypic, genotype-specific protective immunity. Both of the current RV vaccines are based on the idea of cross-

protection: the first RV infection offers substantial protection, and the next infections virtually total protection against moderate to severe RV AGE, regardless of the causative RV type. A range of genotypes other than the major Western-world genotypes and other than the genotypes included in the RV vaccines are seen, for example, in Africa and in Asia. However, the efficacy of RV vaccines against these genotypes seems to be similar to that against the usual genotypes (300).

In pediatric practice, the change that the universal RV vaccination has brought has been astonishing: while in the past, during the busiest RV seasons almost half of the pediatric infectious wards were occupied with children having rehydration therapy because of AGE, in the seasons following the introduction of the vaccine in the NIP, AGE was rarely seen in these wards.

6.6 Future perspectives

The epidemiology and burden of RV AGE in children is quite well known to date. The number of countries that have adopted these vaccines in their NIP is increasing, and the burden of RV AGE is declining. Further follow-up is needed, however, to add to the data on circulating and potentially emerging RV genotypes. It is also essential to follow the efficacy of vaccines in the target population in the long run.

It is very important to continuously follow up on the adverse effects of RV vaccines, as well as those of other vaccines. Serious adverse effects have been rare: studies of intussusception associated to RV vaccines estimate the number of excess intussusception cases to be 1-5 per 100 000 vaccinated infants (282,283,285). No other severe adverse effects have been reported.

The importance of NoV as a causative agent of AGE in children was emphasized in the 2000s. In addition to the AGE epidemics and outbreaks in people all ages, the burden of seasonal NoV AGE in children has been widely recognized. In the aggregate, the burden of NoV AGE rose in the 2000s, along with emergence of the new, highly transmissible and quickly evolving NoV genotype GII.4, which has developed new pandemic variants in about every two years. Surveillance of NoV epidemiology is essential, and global follow-up and data sharing systems have been set up (NoroNet, CaliciNet). The majority of this surveillance, however, is targeted at outbreaks. In Finland, fast diagnostic tools based on rapid PCR for both AGE and other pediatric infections are becoming a

part of clinical practice, and NoV infections can be recognized more readily. These methods detect the NoVs usually up to the level of genogroup, and if more specific recognition is needed, for example for epidemiologic reasons, central laboratories such as THL can be used to study them more precisely.

There are some practical problems with RV vaccines that can limit their use, especially in developing countries. The vaccine doses should be administered between the ages of 6 and 24 weeks (using Rotarix®) or 32 weeks (using Rotateq®) to ensure efficacy and safety. If the closest vaccination center is far away, children may not be vaccinated. The live vaccines cannot be given to immunocompromised children, even though they probably would be safer for them than the natural infection.

One interesting target of research is RV VP6 protein vaccines. This protein of the inner RV capsid is highly antigenic and conserved, whereas the outer capsid proteins VP4 and VP7 have a wide antigenic variation (132,328). A recombinant protein vaccine would be safe also for immunocompromised persons.

There is a definite need for a vaccine against NoVs as well. After elimination of RV AGE, the incidence of NoV was not affected. Even before RV immunization, in some seasons NoVs caused just as many AGE cases seen at hospital level as RVs did, and it can be assumed that at least as many NoV cases as RV cases were among the cases who were treated at the primary care level or did not seek any medical help. In the Western countries, in which RV vaccines are widely used, the NoVs are now the most important cause of AGE hospitalizations in children. Not only children are prone to AGE: NoVs cause annual AGE outbreaks especially in geriatric hospitals and elderly homes: outbreaks that are very hard to control, lead to a high level of morbidity and result in a great deal of suffering and high cost.

Because of the naturally occurring escape of immunity of NoVs and their continual evolution, it is not easy to find an efficient vaccine that provides sustained immunity. Today, many research groups are targeting vaccines against NoVs (132,170,332). A cost-effective and practical approach in immunizing against NoVs would probably be a non-live combined RV-NoV vaccine. One such promising vaccine, which includes recombinant baculovirus-derived NoV GII.4 and GI.3 VLPs and RV recombinant VP6 protein, is now in Phase I studies at the Vaccine Research Center in Tampere (132,328). Combining vaccines against two major AGE-causing viruses would make the vaccination practical and probably reduce the price as well.

Aside from children, elderly people would be a good target for both NoV and RV vaccines, due to their susceptibility to AGE because of weakened immunity,

and their general vulnerability to illness. However, for the same reason (the weakened immune response) that the elderly are prone to infections, immunizing them with vaccines is often difficult to achieve. The herd immunity achieved by vaccinating children might at least offer partial protection for the elderly as well.

7 Conclusions

At the time of the study, before the implementation of universal RV vaccination in Finnish NIP, RVs were the major causative agents of AGE in children in Finland causing the largest part the AGE episodes in which hospital treatment was needed. The second most important causative agents were NoVs.

Evaluating the severity of AGE by a 20 point severity scale, RVs caused the most severe AGE episodes in children, but AGE cases caused by NoVs were also of high severity: fever in RV AGE was higher, and a trend of other symptoms being more severe in RV AGE than in NoV was seen. Both RV AGE and NoV AGE showed a winter seasonality, but the seasons were independent of each other. The dominant RV genotype overall was G1P[8] and the dominant NoV genotype was GII.4.

The costs of AGE are remarkable for both society and families. The annual costs of RV AGE in children in Finland (before introduction of universal vaccination against RV) were estimated to be about €3 700 000, and those of NoV AGE about €1 800 000.

The AGE cases associated to Pirkanmaa waterborne AGE outbreak were analyzed as a separate study. As a conclusion, heavily contaminated drinking water caused unusually severe AGE episodes, in which many different combinations of AGE causative agents were simultaneously found from children.

The study material obtained in 2006–2008 was subsequently used as baseline for evaluation of the effect of universal RV vaccination of children in Finnish NIP from 2009 to 2011 with similar methods to the earlier one. After implementation of the vaccine, the RV AGE seen in Tampere university Hospital declined by 80%. No change in the epidemiology of NoV AGE was seen after RV vaccinations.

8   Acknowledgements

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Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak

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SUMMARY

We examined stool specimens for viral pathogens from 50 children referred to hospital due to acute gastroenteritis (AGE) resulting from consuming drinking water contaminated with sewage in a Finnish community using PCR methods. Rotavirus was detected in 33 (66%), human calicivirus in 31 (62%), and both in 40% of cases. Of the caliciviruses, 20/31 (65%) were noroviruses and 11 (35%) sapoviruses. Furthermore, Aichi virus was detected in 25 (50%), adenovirus in six (12%) and bocavirus in four (8%) cases. *Campylobacter jejuni* was present in 20 (61%) and *Salmonella* in four (12%) of the 33 stools cultured for bacteria. On a 20-point scale median severity score of AGE in the 28 hospitalized children was 17; the severity was similar regardless of viruses detected. Bloody diarrhoea occurred only when *C. jejuni* was present. To conclude, massive exposure to several AGE viruses caused mixed infections and severe AGE regardless of the aetiological agents.

Key words: Community outbreaks, gastrointestinal infections, Norwalk agent and related viruses, paediatrics, rotavirus.

INTRODUCTION

In developed countries gastroenteritis viruses cause both seasonal acute gastroenteritis (AGE) and occasional outbreaks associated with contaminated food or water. In children, rotaviruses (RVs) are the most common causative agents of seasonal AGE, but are infrequently associated with outbreaks [1–3]. RVs cause more severe AGE than other viruses, more commonly requiring rehydration and hospitalization [4–6]. Human caliciviruses (HuCV) include noroviruses (NoV) and sapoviruses (SaV). NoVs are the second most common causative agent of seasonal AGE in young children in Finland [7] and elsewhere [8, 9]. In outbreaks of AGE, NoVs are the most common causative agents in both children and adults worldwide [1, 3, 9, 10]. NoV genotype GII.4 has recently emerged as the most common and virulent type [11–13]. SaVs also cause seasonal AGE in children, even though the clinical picture is milder than that of NoVs [7, 8, 10]. SaVs are rarely reported in outbreaks [9, 14].

Adenoviruses (AdVs) belonging to group F (types 40 and 41) may also cause AGE in both children and adults in about 1–8% of endemic cases and outbreaks [3, 15, 16]. Aichi viruses (AiVs) have been detected with low frequency in children and adults, mainly in AGE outbreaks [17–19]. Since AiVs have often been

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found together with other AGE viruses, they might even be considered to be indicators of mixed infections [18]. Human bocaviruses (HBoVs) were first recognized in respiratory infections in children, but have recently also been connected with AGE in children [20–22].

An outbreak of AGE due to contamination of drinking water with sewage occurred near Tampere in late 2007. We investigated cases of AGE in children from the contaminated area seen in Tampere University Hospital between 28 November and 31 December 2007, and examined the stool specimens for various causative agents.

MATERIALS AND METHODS

Drinking water was contaminated with treated sewage on 28 November 2007 in Nokia, a town of about 30 000 inhabitants near Tampere in southern Finland. It was estimated that thousands of people had symptoms of AGE, and at least 758 patients visited public health centres [23] in the following days and weeks. A total of 115 children requiring rehydration therapy were referred to Tampere University Hospital.

Study subjects

At the time of the incident, we were conducting an epidemiological survey of AGE in children in Tampere University Hospital. The study protocol and consent forms had been approved by the Ethics Committee of the Pirkanmaa Hospital District in 2006. The children treated in the hospital because of the Nokia AGE outbreak were recorded as a subgroup of this epidemiological study.

According to the study protocol, all children aged <15 years presenting with AGE symptoms in Tampere University Hospital were eligible for enrolment. The parents of eligible children ($n = 115$) were informed about the study and asked to sign an informed consent form. We enrolled 65 children and obtained stool samples from 50 cases; of these 28 were hospitalized and 22 were treated as outpatients.

We collected clinical information on each AGE episode, including date of onset of symptoms, frequency of vomiting and diarrhoeal stools, and fever. The information was derived from a questionnaire completed by the parents, and from the hospital records. Some missing information was obtained by telephoning the parents. We also collected information on whether the child had received one or more RV vaccines.

A 20-point scoring system [24] was used to assess the severity of AGE episodes.

Laboratory methods

RVs, HuCVs, AdVs, HBoVs, and AiVs were primarily detected with the respective PCR methods. All positive amplicons were sequenced to confirm the result and to determine the virus genotype.

All 50 stool specimens were tested for RV G-types by reverse-transcription (RT)–PCR, as previously described [7], with the modification that the *Taq* polymerase was replaced by GoTaq® polymerase (Promega, USA). Two different primer sets were used for G-typing (see [25, 26]). For P-typing, an RT–PCR method previously described [27] was used with modified primers and conditions. The presence of RV antigen in stools was detected with ELISA, using IDEIA® Rotavirus kit (Oxoid Ltd, UK) according to the manufacturer's protocol. Five stool samples were from nappies only and could not be tested by ELISA.

HuCVs were detected by a modified RT–PCR method [28, 29]; the primers co-detect NoVs and SaVs. The presence of AiVs was ascertained by a nested PCR after separate RT reaction with random primers [10]. The AiV-detecting PCR method was originally developed by Yamashita *et al.* [30]. AdVs were detected by a previously described nested PCR [31] using two primer sets and HBoVs were detected using a PCR-detecting HBoV1 [32].

Enteropathogenic bacteria were studied in 33/50 stool samples by bacterial culture, which detects *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia*, *Aeromonas*, and *Plesiomonas*. The cultures were performed in the microbiological laboratory of Tampere University Hospital as part of routine examinations and not as part of the study protocol.

RESULTS

Of the eligible 115 children, 35 (30%) were hospitalized and 80 (70%) were treated as outpatients with one or more visits. We enrolled 65 children; 28 were hospitalized and 37 treated as outpatients. Stool specimens were obtained from all 28 hospitalized

Table 1. *Findings of viral and bacterial pathogens and clinical characteristics of the 28 children hospitalized for acute gastroenteritis during the outbreak*

Case	Sex	Age	Score	Pathogen(s)	Duration of diarrhoea (days)	Max. no. diarrhoeal stools/24 h	Duration of vomiting (days)	Max. no. vomiting episode/24 h	Fever max. (°C)
1	F	2 yr 4 mo.	19	RV, SaV, AiV, C.j., Salm.	6	10	3	10	38·5
2	F	3 yr 0 mo.	19	RV, NoV, C.j.	19	>10	4	10	39·1
3	M	23 mo.	16	RV	5	20	1	10	39·1
4	M	2 yr 2 mo.	17	RV, SaV, AiV, C.j.	>12	13	5	>6	38
5	F	10 mo.	17	NoV, C.j.	12	10	8	5	38·2
6	F	10 mo.	17	RV	19	5	2	10	39·6
7	M	8 yr 3 mo.	15	RV, NoV, AiV, C.j.	>3	>7	6	>8	37·7
8	M	8 yr 10 mo.	15	RV, AiV, C.j.	>3	10	6	12	37·7
9	M	16 mo.	16	RV, NoV, AdV, C.j.	8	>10	4	3	38
10	F	4 yr 4 mo.	19	RV, SaV, AdV, C.j.	7	10	5	6	38·5
11	M	11 yr 10 mo.	19	RV, AiV, C.j.	>10	>8	9	>8	38·8
12	F	7 yr 5 mo.	17	RV, AiV, HBoV, C.j., Salm.	12	>8	4	>10	38
13	M	10 mo.	18	RV, NoV, C.j.	16	8	10	8	38·5
14	M	18 mo.	16	RV, AiV	9	15	1	4	39
15	F	10 yr 3 mo.	13	RV, NoV, AiV	4	1	6	10	38
16	F	13 yr 0 mo.	14	RV, SaV, AiV	8	n.a.	6	>7	37·7
17	M	9 yr 0 mo.	17	SaV, AiV, C.j.	13	20	2	3	40
18	F	7 mo.	18	RV, NoV, AiV, C.j.	17	>8	5	>8	38·5
19	F	9 mo.	12	RV, NoV	>6	5	>5	2	39·4
20	F	2 yr 5 mo.	18	RV	7	7	2	10	40·4
21	M	6 yr 1 mo.	17	RV, SaV	5	>10	>6	10	n.a.
22*	M	14 mo.	15	RV, NoV	14	15	3	2	No fever
23	F	7 yr 5 mo.	20	RV, NoV, AiV, AdV, HBoV, C.j., Salm.	14	10	11	>8	39·5
24	M	9 mo.	17	RV, AiV	9	>10	9	>15	38
25*	F	8 mo.	19	SaV, AiV, C.j.	>20	10	5	3	40
26	M	5 yr 5 mo.	7	RV, NoV, AiV, AdV	2	3	1	3	No fever
27	M	14 mo.	15	RV	>7	>6	4	>5	n.a.
28*	M	10 mo.	14	NoV	>3	10	8	2	37·8

RV, Rotavirus; SaV, sapovirus; AiV, Aichi virus; NoV, norovirus; AdV, adenovirus; HBoV, human bocavirus; *C.j.*, *Campylobacter jejuni*; *Salm.*, *Salmonella* species; n.a., information not available.
* RV-vaccinated children.

children and from 22/37 children treated as outpatients. All 50 stool specimens were tested for all viruses (RV, HuCV, AiV, AdV, HBoV).

Of the 50 stool specimens 33 (66%) were RV positive, 31 (62%) were HuCV positive, five (10%) were AdV positive and 25 (50%) were AiV positive. In 20 (40%) cases both RV and HuCV were found; 10 (20%) cases presented with a third virus, and in two (4%) cases four viruses were present. Bacterial culture was performed on 33/50 cases; *C. jejuni* was found in 20/33 (61%) and *Salmonella* sp. in 2/33 (6%) of the cases. The complete findings of viruses and

bacteria in the 28 hospitalized cases are given in Table 1.

Rotaviruses

RV antigen ELISA in stools was positive in 24/45 (53%) cases tested, and RV RT–PCR in 33/50 (66%) cases. All stool samples which were RV positive by ELISA were also positive by RV RT–PCR. Five stool specimens were negative for RV antigen by ELISA, but positive for RV by RT–PCR. Of the 33 RT–PCR-positive RV cases, 32 were of genotype G1P[8] and

Table 2. *Combinations of viruses detected in the 50 cases of acute gastroenteritis enrolled in the study during a waterborne outbreak*

Virus	RV	NoV	SaV	AdV	AiV	HBoV		n
One virus	+							6
		+						5
					+			2
						+		1
							Total	14
Two viruses	+	+						5
	+		+					3
	+				+			6
		+			+			3
			+		+			2
							Total	19
Three viruses	+	+		+				1
	+	+			+			4
	+		+	+				1
	+		+		+			4
	+				+	+		1
							Total	11
Four viruses	+	+		+	+			1
			+	+	+	+		1
							Total	2
Five viruses	+	+		+	+	+		1
							Total	1
No viruses							Total	3
Total								50

RV, Rotavirus; SaV, sapovirus; AiV, Aichi virus; NoV, norovirus; AdV, adenovirus; HBoV, human bocavirus.

one was of genotype G4P[8]. All the RVs of genotype G1 were identical, meaning that $>99\%$ of sequenced nucleotides encoding for VP7 were identical (length of amplicon 749 bp).

RV was the only identified viral AGE pathogen in six (12%) stool samples. RV presented with NoV in five (10%), with SaV in three (6%), and with AiV in six (12%) stool samples. Several different combinations of mixed infections with multiple viruses were found (see Table 2).

Noroviruses and sapoviruses

RT–PCR for HuCV was positive in 31/50 (62%) cases. Of the 31 HuCVs, 20 (65%) were NoVs and 11 (35%) were SaVs.

Altogether, eight different genotypes of NoV were identified. Of the NoVs, 19/20 belonged to genogroup GII and only one to genogroup GI. Of genogroup GII NoVs, 12 (63%) were genotype GII.4. Of the 11 SaVs, eight different genotypes were found. In five (10%) of

all 50 cases NoV was the only viral pathogen found. There were no cases in which SaV was the only viral pathogen. HuCVs presented together with RV in eight cases; in five cases NoV with RV, and in three cases SaV with RV. AiVs, AdVs and HBoVs were also detected in mixed infections with HuCVs (Table 2). There were no cases in which more than one type of HuCV was present.

Adenoviruses

AdV was found in five (10%) of the 50 cases. Two of these were of group A, one of group C, and two of group F. All the AdV-positive cases occurred in mixed infections (Table 1).

Aichi viruses

AiV was present in 25/50 (50%) stool samples. In 24/25 (96%) cases AiV was found in mixed infections; in one case no other AGE viruses were found (Table 1).
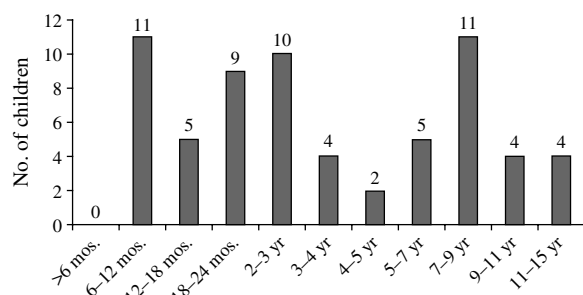
Fig. 1. Age distribution of 65 children seen in Tampere University Hospital and enrolled in the study during the waterborne outbreak of acute gastroenteritis.

Fig. 2. Mean clinical severity scores in 28 children hospitalized for acute gastroenteritis according to causative viruses. RV, Rotavirus; SaV, sapovirus; AiV, Aichi virus; NoV, norovirus; AdV, adenovirus; HBoV, human bocavirus.

The median age of the AiV-positive cases was 7 years, i.e. higher than the median of all the enrolled children (see Clinical features below).

Human bocaviruses

HBoV was found in four (8%) stool samples. In one case HBoV was the only pathogen identified in the stool sample. In the other three cases, HBoV presented as a part of a mixed infection.

Bacteria

Bacterial cultures were performed for 33/50 cases. *C. jejuni* was found in 20/33 (61%) cases and *Salmonella* sp. in four (12%) cases. In each case these bacteria were found in mixed infections with one or more AGE viruses. *C. jejuni* was found in 13/33 RV-positive stool samples, in three of which *Salmonella* sp. was also present. *C. jejuni* was found in 15/31 HuCV-positive cases, in three of which *Salmonella* sp. was also present. In five (10%) cases *C. jejuni* presented with RV, HuCV and AiV, and in one case all the pathogens studied (RV, HuCV, AiV, AdV, HBoV, *C. jejuni* and *Salmonella* sp.) were present.

RV vaccination status

There were three children who had been vaccinated against RV. One had been vaccinated with Rotarix[®] (GlaxoSmithKline, Finland), one with Rotateq[®] (Sanofi Pasteur MSD, Finland), while in the third case the vaccine was unknown. In all of these cases ELISA for RV antigen was negative, but in one case a weak positive RV-type G1 was detected with RT–PCR. In this case the RV was of wild-type G1 virus similar to the other G1 RVs found in this outbreak, not of the vaccine type. In the remaining

two cases RV RT–PCR was clearly negative. In all three cases other pathogens were found: NoV genotype GII.4 in two cases, and SaV, AiV, AdV, and *C. jejuni* in one case.

Clinical features

The age of enrolled children ranged from 6 months to 13 years (median age 2 years 6 months). There were 19 children aged >7 years (Fig. 1).

The median severity score of the hospitalized children (n = 28) was 17 (Fig. 1), indicating an unusually severe disease. However, no difference in the severity of AGE was observed between the cases caused by a single virus, different combinations of viruses, or combinations of viruses and bacteria (Fig. 2, Table 1). The mean duration of diarrhoea was 10·3 days (range 2 to >20 days). The mean duration of vomiting was 5 days (range 1–11 days). The mean of maximal fever was 38·7 °C (range up to 40·4 °C) (Table 1).

Considering single symptoms, the only significant difference between the various pathogens or combinations of pathogens was that bloody diarrhoea occurred only if *C. jejuni* was found in the stool samples. Altogether four children had bloody diarrhoea, and in each of these cases a combination of pathogens including *C. jejuni* and RV was found.

DISCUSSION

We were able to enrol in the study and collect stool samples from 50/115 children (43%) seen in Tampere University Hospital because of this outbreak, including 28/35 hospitalized children (80%). The main reason for failing to enrol more patients was that the emergency room was crowded during the outbreak,

and many of the children were discharged before parental consent could be obtained. However, the study material still represents almost half of all the children who were seen in the hospital during this outbreak, and 80% of the hospitalized children. Because our study is hospital-based, the study material includes severe AGE cases rather than being representative for all paediatric AGE cases in the outbreak. Moreover, children were referred to this hospital whenever rehydration was required, effectively selecting the severe cases in children.

This AGE outbreak might be seen as an experimental situation in which many children were simultaneously, and massively, exposed to enteric pathogens. It is impossible to estimate the infectious dose or proportion of each pathogen, but presumably children with severe AGE symptoms were exposed to unusually large doses of several gastroenteritis viruses. As a result, AGE episodes seen in the hospital were unusually severe and enduring. Furthermore, children of all ages suffered from AGE, suggesting that the viral pathogens were able to break through the existing immunity in older children.

The majority of the symptomatic children had multiple AGE pathogens simultaneously. Regardless of the pathogen or combination of pathogens, the AGE symptoms of the hospitalized children were unusually severe, but did not differ for individual pathogens. This is in contrast to the normal endemic or epidemic situation. For example, RV generally causes more severe vomiting, diarrhoea and fever than other viral pathogens [4, 5, 8], and NoVs typically cause more vomiting than diarrhoea, whereas SaVs cause more diarrhoea then vomiting [7, 8]. No such features were seen in this study. Only one child had a severity score <11, usually regarded as a threshold for severe AGE. This male child had mild AGE symptoms, but developed severe post-infectious abdominal pain, which caused a suspicion of intestinal intussusception. NoV type GII.4 and AdV were found in his stool sample, but intussusception was excluded.

Most of the RVs and many NoVs showed genetic similarity. Of the 33 RVs, 32 G1 viruses were genetically 99% identical. While several HuCVs were found, NoV genotype GII.4 detections showed particular genetic similarity. These findings suggest a single common source of G1 RVs and GII.4 NoVs in this outbreak. When the contamination of drinking water occurred, the endemic RV season was just beginning, and a single transmitter of G1 RV might explain all but one of the RV findings. In contrast, some NoV-associated AGE had also already been seen in the hospital throughout autumn 2007, explaining the greater variety of NoVs at the time of the outbreak. Both NoVs and RVs are highly infectious, and even a few viruses are able to cause infection and disease [33, 34].

RVs only rarely cause waterborne or foodborne outbreaks. It can be assumed that in countries with a high level of hygiene, the infectious dose of RVs from either person-to-person contact or via airborne infection is probably small. Compared with such a background, the children in this outbreak may have received a much larger amount of RVs from the contaminated drinking water, which would explain the unusually severe clinical picture, as well as the occurrence of RV cases in older children.

As discussed earlier for RVs, the infectious dose of NoVs is probably small in endemic seasonal infections or in usual foodborne or waterborne outbreaks. Usually NoV-associated AGE cases in such epidemics are less severe than those seen in this outbreak [7, 35]. SaVs usually cause milder diarrhoea in children aged <5 years [7, 8]. In previous studies on all these viruses, severity scores in prospectively followed-up cases in children are in the range of 8–11, regarded as moderately severe, and in extremely rare cases exceed 17, which was the mean severity score in the present situation. We believe that simultaneous exposure to several pathogens on the one hand and massive exposure on the other, explains the unusually severe clinical picture in these cases.

This is the first AGE outbreak in which AiVs have been found in Finland. In our material, AiV was found in 25/50 (50%) stool samples; 24/25 were in mixed infections. Our finding confirms the earlier notion that AiVs usually present together with other AGE pathogens in mixed infections [18].

The role of HBoVs as causative agents in gastroenteritis is unclear. These viruses have been detected in stool samples of AGE cases and also in respiratory infections [20, 36], but in stool samples they are usually seen in mixed infections. In this outbreak, four cases with HBoV detected in stool samples were seen, of which in one case HBoV was the only pathogen identified. In this case HBoV may have been the causative agent in AGE.

In our study there were three children, who were known to have received RV vaccine. In all three cases the AGE symptoms were severe enough (scores of 14, 15, 19) to lead to hospitalization. In all three cases,

HuCVs were presumed to be the main causative agent. NoV type GII.4 was found in two cases and SaV together with AiV, AdV, and *C. jejuni* in one case. One of the children with NoV GII.4 had a weak positive RV by RT–PCR, but no detectable RV antigen by ELISA in his stool sample, meaning that the amount of RV in the sample was small. This finding suggests that vaccination probably protected this child, and possibly the others, from RV AGE and only minimal replication of wild-type RV occurred in this one case.

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DECLARATION OF INTEREST

None.

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ORIGINAL ARTICLE

Rotavirus gastroenteritis in Finnish children in 2006–2008, at the introduction of rotavirus vaccination

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Abstract

Rotaviruses (RV) are major causative agents of acute gastroenteritis (AGE) requiring hospitalization in children; RV hospitalizations may be largely eliminated by universal mass vaccination with RV vaccine. We conducted a hospital-based prospective survey of AGE in children over 2 RV epidemic seasons, from 2006 to 2008, when the coverage of RV vaccination in Finland increased to 35% of the birth cohort. RVs were detected by reverse transcription polymerase chain reaction (RT-PCR). In the first season, only 38% of AGE cases were RV-positive, and the onset of the RV season was delayed. Type G1P[8], RVs accounted for 40%, G2P[4] for 19%, G3P[8] for 2%, G4P[8] for 2% and G9P[8] for 38%. In the second season, 63% of AGE cases were RV-positive: G1P[8] accounted for 73%, G2P[4] for only 3%, G3P[8] for 4%, G4P[8] for 13%, and G9P[8] had almost disappeared. G2P[4] RV did not become predominant at the coverage level of 29% of G1P[8] human RV vaccine. RV-associated hospitalizations were seen in children up to the age of 9 y. This study forms the epidemiological background for the follow-up of the impact of universal RV vaccination in Finland introduced in 2009.

Introduction

Rotaviruses (RV) are major causative agents of acute gastroenteritis (AGE) in children worldwide [1]. In Europe RVs are responsible for up to two-thirds of AGE hospitalizations and outpatient visits [1–4].

G1P[8] is the predominant RV genotype in Europe, North America and Australia [5–9]. However, other G-types such as G2, G4, and, more recently G9, have emerged and even become predominant in some y and regions [6,7,9,10]. In Europe, the proportion of G9 has increased since the first reports were published in the 1990s [11] to up to 90% of the seasonal RV infections [6,10]. G9 strains present most often with P-type P[8], but may also combine with other P-types. Other new combinations of the common G- and P-types either with each other, or with emerging G- and P-types, are also increasingly seen globally [8,12]. Seasonal fluctuation in the prevalence of RV genotypes also occurs within regions [6,7,9]. In Europe, a surveillance network, EuroRotaNet, was established in 2007 to gather and provide information on RV epidemiology and strain diversity [6].

Two live oral attenuated vaccines against RVs have been available since 2006. One is derived from human RV type G1P[8] (Rotarix®, GlaxoSmithKline) and the other from WC-3 bovine rotavirus reassorted with RV G-types G1–G4 and with P-type P[8] (RotaTeq®, Sanofi Pasteur MSD). Both vaccines have been found safe and effective in Europe and elsewhere [13,14]. The long-term consequences of universal RV vaccinations may include the emergence of new RV serotypes not covered by the vaccines or against which the vaccines are known to be least effective, especially G2P[4] [15,16], and possibly postponement of RV cases to older age groups. In Brazil, before the start of mass vaccinations against RV in 2006, the most common RV genotype was G1P[8] (43%), followed by G9P[8] (22%) and G2P[4] (7%) [17]. After the introduction of a universal RV vaccination programme, a survey undertaken in a Brazilian city with 51% vaccine coverage found that all the RV-positive AGE cases seen in a hospital were of type G2P[4] [15].

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This study was conducted to determine the prevalence of RV AGE in hospitalized children and the proportions of RV genotypes prior to the introduction of universal rotavirus vaccination in Finland, anticipated to begin in 2009.

Materials and methods

Clinical methods

The study was approved by the Ethics Committee of Pirkanmaa Hospital District and conducted at Tampere University Hospital from September 2006 to August 2008. Approximately 80,000 children aged <15 y live in Pirkanmaa Hospital District. More than half come from urban areas in Tampere or smaller surrounding communities, and less than half from rural areas. All children in need of paediatric emergency room (ER) care or hospitalization are referred to Tampere University Hospital; no rehydration therapy for AGE is usually given in communal health centres or private clinics.

Children ≤15 y of age, either seen in the ER or admitted to the hospital with AGE, were eligible for enrolment. Prior to enrolment, a parent or legal guardian provided written informed consent for participation.

Parents were interviewed about their child's symptoms before the hospital visit and about their child's rotavirus vaccination status. A stool specimen was collected in the ER or on the hospital ward.

If the child had required more than 1 ER visit or hospitalization due to AGE during the study period, the visits were considered to represent 2 separate episodes if there were more than 7 symptom-free days between them.

We also collected information on all the AGE patients aged ≤15 y seen in the hospital during the study period from an independent source. For this purpose all ICD-10 diagnoses of groups A01–A09 were retrieved.

Open access data on the number of doses of the RV vaccines sold during the study period from September 2006 to August 2008 were obtained from GlaxoSmithKline and Sanofi Pasteur MSD. From the monthly number of vaccine doses sold in the whole country, we estimated the number of children who had received full vaccination courses of either vaccine, and calculated the coverage of RV vaccination per birth cohort in each y and each season. The number of live births was obtained from Statistics Finland.

Laboratory methods

All stool samples were tested for RVs using a reverse transcription polymerase chain reaction (RT-PCR) method, as described by Pang et al. [18], with the modification of replacing Taq polymerase with GoTaq polymerase (Promega, Madison, WI, USA). For the determination of RV G-types, 2 different primer sets were used: those introduced by Gouvea et al. [19] and those by Das et al. [20]. All the RV VP7 amplicons were sequenced to confirm the G-type findings.

For the determination of RV P-types, an RT-PCR method to amplify the VP4 genome segment introduced by Simmonds et al. [21] was used with some modifications. Reverse primers in the second round PCR reaction were the same for P[4] and P[8], but for P[6] and for the forward primer, modified primers were used (5′-GATGGTCCDTATCARCC-3′ forVP4 fwd and 5′-ATTTGAAGTTGACGAGTA-3′ for P[6]). The RT-PCR assay detected P-genotypes P[4], P[6], and P[8].

The presence of RV antigen was also detected in stool samples with an enzyme-linked immunosorbent assay (ELISA), using the IDEIA Rotavirus kit (Oxoid Ltd, UK), unless the sample had been exhausted in PCR. Stools absorbed in a diaper were not tested for RV antigen, because the ELISA test could technically not be performed.

Results

A total of 1723 patients were seen for AGE at Tampere University Hospital during the 24-month period. Of these, 1193 were recruited (69% of eligible subjects). Stool samples could be obtained from 809 children (68% of those recruited); of these 341 stool samples were collected in the first RV season (defined as September 2006 to August 2007) and 468 samples in the second season (defined as September 2007 to August 2008). The proportions of RV-positive cases in the results are counted out of the 809 cases from which the stool samples were available.

Of all 809 cases of AGE, 421 (52%) were RV-positive. Of these, 54% were hospitalized and 42% were treated as outpatients (Figure 1). Of the RV-positive cases on the hospital ward, 4% were nosocomial infections. Of the RV-negative cases, 49% were hospitalized and 51% treated as outpatients.

Among 305 RV RT-PCR-positive cases that could be tested by enzyme immunoassay (EIA), 278 (91%) were positive and 27 (9%) were negative for RV antigen. One hundred and sixteen stool samples were not tested for RV antigen because the stool was in a diaper ($n = 102$) or the whole sample had been exhausted in RT-PCR studies ($n = 14$).

In the first y (2007 season), RV was found in 128 (38%) of 341 stool samples obtained, and in the second y (2008 season) in 293 (63%) of 468 stool samples obtained. In the first RV epidemic season, the
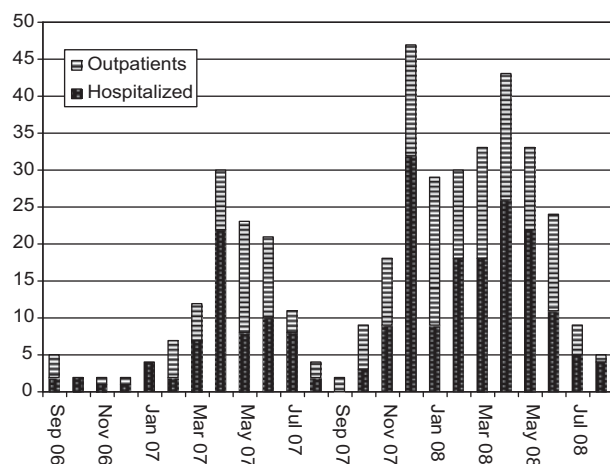
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Figure 1. Timing of the rotavirus seasons between September 2006 and August 2008 and treatment needed in the 421 children seen at Tampere University Hospital because of acute rotavirus gastroenteritis.

majority of RV-positive AGE cases were seen between April 2007 and June 2007, i.e. relatively late, whereas in the second season the most active months were between December 2007 and April 2008.

The proportion of RV in all the enrolled AGE cases seen as outpatients was 33% in the first y and 62% in the second y. The proportion of RV in the AGE cases admitted to hospital was 43% in the first y and 66% in the second y.

The age distribution of RV-positive cases is shown in Figure 2. Of the 421 RV-positive cases, 233 (55%) were between ages 6 and 24 months, and more specifically, 149 (35%) were between ages 12 and 24 months. Only 20 (5%) of the RV-positive children were younger than 6 months. On the other hand, 37 (9%) RV-positive AGE cases were in children over 5 y of age. Of these, 12 children had caught RV as a result of an AGE outbreak caused by drinking water
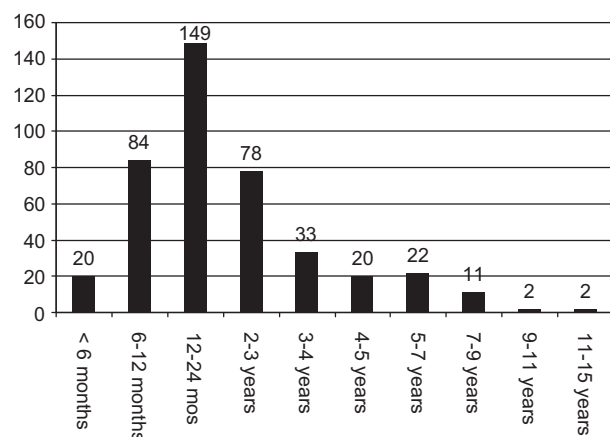

Figure 2. Age distribution of 421 children treated at Tampere University Hospital between September 2006 and August 2008 because of rotavirus gastroenteritis.

contamination in the town of Nokia in 2007 [22], but 24 were other community-acquired cases. Of the 24 children, 18 (75%) were hospitalized and 6 (25%) treated as outpatients.

All the common RV genotypes, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], were seen in both seasons (Table I). In the first season ($n = 128$), genotypes G1P[8] ($n = 51$, 40%) and G9P[8] ($n = 49$, 38%) were predominant. Type G2P[4] was found in 24 (19%) cases. In the second season ($n = 293$), G1P[8] ($n = 214$, 73%) was the predominant strain. Type G2P[4] was found in 9 (3%) and G9P[8] in 2 (1%) cases. There were 4 cases in which more than 1 RV type was found in stools simultaneously: in 2 cases G1P[8] with G3P[8] and in 2 cases G1P[8] with G4P[8]. RV G-types G8 and G12 were not found.

One uncommon RV type with the closest resemblance (96% in the 350-bp amplicon) to rhesus RV strain was found. The closest human RV match was 85% in the 350-bp amplicon. This unusual RV was found in connection with the waterborne outbreak in the town of Nokia in November 2007.

In 4 stools there were 2 VP4 types, P[8] and P[4], found with VP7 type G1. In 8 cases the P-type was not determined: the P-type was not detectable with RT-PCR in 5 stool samples, and in 3 cases it could not be determined because the specimen was exhausted before P-type studies.

Among the 1193 AGE cases enrolled, there were 49 (4%) children who were known to have received at least 1 dose of either of the RV vaccines. A stool specimen was obtained from 36 of these children and RV was found in 8 specimens. In 3 of these cases, vaccine type G1P[8] RV and in 4 cases wild-type G1P[8] were found in the stools. One specimen

Table I. Rotavirus genotypes in the 421 rotavirus-positive cases in children with acute gastroenteritis, Tampere University Hospital, September 2006 to August 2008.

Virus genotype	1st season (%)	2nd season (%)	All
G1P[8]	51 (39.8)	214 (73.0)	265 (62.9)
G2P[4]	24 (18.8)	9 (3.1)	33 (7.8)
G3P[8]	2 (1.6)	13 (4.4)	15 (3.6)
G4P[8]	2 (1.6)	38 (13.0)	40 (9.5)
G9P[8]	49 (38.3)	2 (0.7)	51 (12.1)
G1, P[4] and P[8]	0	4 (1.4)	4 (1.0)
Mixed G1P[8] and G3P[8]	0	2 (0.7)	2 (0.5)
Mixed G1P[8] and G4P[8]	0	2 (0.7)	2 (0.5)
Rhesus rotavirus	0	1 (0.3)	1 (0.2)
G1, P undefined[a]	0	4 (1.4)	4 (1.0)
G3, P undefined[a]	0	4 (1.4)	4 (1.0)
Total	128 (100)	293 (100)	421 (100)

[a]In 5 stool samples (2 stools with G1 and 3 with G3), the P-genotype was indefinable by RT-PCR; in 3 stools (2 with G1 and 1 with G3), the stool sample was exhausted and P-type could not be detected.

contained RV type G3 with non-detectable P-type. Only 1 case with wild-type RV (case patient 8 in Table II) was clearly positive both in RT-PCR and in EIA (Table II). Of the 36 stool specimens, 14 were positive for norovirus and 1 was positive for sapovirus.

RV vaccines have been available in Finland since May 2006. The first vaccine available was live attenuated human RV vaccine (Rotarix; GlaxoSmithKline, Finland), and live human–bovine reassortant rotavirus vaccine (RotaTeq; Sanofi Pasteur MSD, Finland) became available from April 2007. At the end of the first RV epidemic season of our study, RV vaccines had been sold corresponding to vaccination coverage of 22% of the birth cohort. In the second season the vaccination coverage increased up to 35% of the birth cohort.

Discussion

In this study, we did not want to examine only age groups, which are commonly discussed with RV AGE, but also wanted to study in which age groups RV AGE is actually seen in hospital. In fact, a significant proportion of the RV AGE cases treated in the hospital were children older than 2 y of age. This was a quite unexpected finding, since most of the former RV studies have concentrated on children under 2 y of age. Along with universal RV vaccination, RV AGE might be seen in older children for some time.

Of the 1723 eligible children, 1193 were recruited. The rest were lost mainly because of the ER being too busy to be able to, or to remember to, recruit all the eligible cases. Furthermore, stool samples were obtained only from 809 of the 1193 recruited children because the stools were collected only while the child was staying in the ER or on the hospital ward. There were many children who spent only a few hours in the ER passing no stools. The possibility of a bias in the AGE-causing agents resulting from these matters cannot be denied, although we could see no relation between the causative agent and the passing

of stools in the ER. However, even with these limitations our study represents almost half (47%) of all the AGE cases seen in children in the hospital over a 2-y period.

Our survey covers 2 very different RV epidemic seasons: one with low RV activity and another with high RV activity. The low RV activity in the first season can hardly be explained by RV vaccinations, because the vaccine coverage was still low at 22%. Interestingly, the onset of the season was delayed, as was reported from the USA and Belgium after the introduction of RV vaccination [23,24]. In these countries the late start of the RV season was explained by RV vaccination, but the case of Finland shows that the season was delayed regardless of this. The second RV season in winter 2008 was more like a 'normal' season, not really different from the early RV studies of 1977–78 [25].

The second season material also included cases of AGE due to an outbreak in November 2007 in the town of Nokia, caused by massive contamination of drinking water with sewage water [22]. During the outbreak, the criteria for referring children to the hospital remained the same as at other times. About 10% of the second season's RV cases can be traced to the Nokia outbreak, and 31 (97%) of them were caused by RV of identical G1-type. However, even excluding these cases, the RV activity in the second season was over twice that seen in the first season.

Interestingly and unexpectedly, in the first season with low RV activity, RV type G9P[8] was common, equal to type G1P[8]: 38% RV-positive cases were of type G9P[8] and 40% were of type G1P[8]. RV type G2P[4] represented 19%, G3P[8] 2%, and G4P[8] 2% of cases. In the second season, however, RV types G2P[4] and G9P[8] were hardly seen, comprising only about 3% and 1% of the RV detections in the season, respectively. With high RV activity in the second season, G1P[8] was clearly the dominant circulating RV type with a 73% share of RV-positive cases. Sudden changes in the balance of the 2 predominant

Table II. Characteristics of 8 acute gastroenteritis cases with a history of rotavirus vaccination, and rotavirus found in the stool specimens.

Case	Age in months	1st day of symptoms	Vaccination date(s)	Vaccine	RV genotype	RV-ag EIA	Other pathogen(s)
1	2	5 Sep 2006	4 Sep 2006	Rotarix	G1 Vac	Pos	–
2	4	12 May 2007	9 May 2007	Rotarix	G1 Vac	Neg	–
3	3	27 Jun 2007	11 Jun 2007	Rotarix	G1 Vac	Neg	–
4	15	2 Dec 2007	Nov 06 and Dec 06	Rotarix	G1 WT	Neg	NoV GII.4
5	14	11 Apr 2008	Unknown	Unknown	G1	Neg	NoV GII.4
6	11	24 Apr 2008	Unknown	Unknown	G3	Neg	NoV GII.4
7	18	14 Jun 2008	Unknown	Unknown	G1	NT	–
8	27	9 Aug 2008	Unknown	Unknown	G1	Pos	–

RV, rotavirus; RV-ag, rotavirus antigen; EIA, enzyme immunoassay; Vac, vaccine type RV; WT, wild type RV; NT, not tested; NoV, norovirus.

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RV types, G1P[8] and G9P[8], in the same follow-up period, were also seen in other parts of Europe, for example in Denmark and in Spain [6]. The emergence of RV types G3P[8] (4%) and G4P[8] (13%) was an unexpected finding of the second season. Since type G2P[4] almost disappeared in the second season, along with rising vaccination coverage, its higher prevalence in the first season is rather suggestive of the random fluctuation of the RV genotypes than a consequence of the vaccinations.

The RV P-type could not be identified in 5 stool samples. In these, the G-type was determined (3 cases of G3 and 2 of G1). All 5 stools were negative for RV antigen by ELISA. In each of these cases norovirus type GII.4 was also found. It is probable that the RV was not the primary pathogen in these cases, but the small amounts present in stools, insufficient for detection by P-type RT-PCR, might possibly have come from an earlier RV infection.

Information on RV G-types in children in Finland is available from a vaccine trial conducted in 2004–2006; i.e. in the 2 seasons prior to the present study (unpublished observations). In this study 155 episodes of rotavirus gastroenteritis (RVGE) were recorded, and of these, 103 cases (66%) were in placebo recipients. In the placebo group in the first season 2004–2005, 51% of the RV cases were caused by G1 wild-type, 26% by G9, 13% by G4, 6% by G3, and 5% by G2. In the second season 2005–2006, 44% of the RV cases in the placebo recipients were G1, 34% were G9, 11% G2, 6% G4, and 3% were G3. The P-serotypes were not determined. These results suggest that non-G1 RVs were already common in the 2 y before the present study.

Our findings are consistent with the findings of the EuroRotaNet surveillance in 15 European countries in 2005–2008, in which the most predominant RV strain was G1P[8] with remarkable diversity of co-circulating common human RV strains in different seasons [6].

The first RV vaccine, Rotarix, became available in May 2006 in Finland. Its coverage reached 22% of the Finnish birth cohort in the first RV season and 29% in the second season. RotaTeq was launched in April 2007 and its coverage reached 6% of the birth cohort by the end of the second season. Altogether, RV vaccination coverage was 22% in the first and 35% in the second RV season in Finland. No variation in the vaccination coverage in different parts of Finland is evident. In the present study material on 1193 children, only 49 (4%) of the children had received at least 1 dose of RV vaccine. Thus we may conclude that the percentage of vaccinated children seen in the hospital outpatient clinic or admitted to hospital because of AGE was lower than the percentage of children vaccinated in the general population

of children of the same age. In fact, only 1 case of AGE (case patient 8 in Table II) showed strong evidence of RV as the causative agent with no other causative agent identified and RV-positive in both RT-PCR and EIA. Calculated from this, the estimated vaccine effectiveness against hospital admission or outpatient clinic visit because of RVGE would be 97%. A rough estimate would suggest about 90% effectiveness for RV vaccination.

Universal RV vaccination with RotaTeq vaccine was introduced in Finland as of 1 September 2009. The impact of the universal mass vaccination will be followed in an epidemiological study with similar designs to the present one, which will serve as a comparison baseline.

Acknowledgements

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ORIGINAL PAPER

Noroviruses in children seen in a hospital for acute gastroenteritis in Finland

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Leena Huhti · Timo Vesikari**

Abstract Noroviruses (NoVs) are second only to rotaviruses (RVs) as causative agents of acute gastroenteritis (AGE) in children. The proportional role of NoVs is likely to increase after control of RV by vaccination. We investigated NoVs in children seen in Tampere University Hospital either treated as outpatients or hospitalized because of AGE before universal RV vaccination was implemented in Finland. This prospective study was conducted from September 2006 to August 2008. A total of 1,128 children <15 years of age with symptoms of AGE were enrolled either in the hospital clinic or in a ward, and stool samples for NoV studies were obtained from 759 children. NoVs were found in 196 (26%) cases. In the first year, NoVs were found in 116 (34%) out of 341, and in the second year, in 80 (19%) out of 418 cases. RVs were found respectively in 128 (38%) and 260 (62%) cases in these two seasons. Both RV and NoV were present in 24 cases. NoV genotype GII.4 predominated with a 96% share of the NoV cases in the first season and an 80% share in the second season. Other NoV genotypes seen infrequently were GII.7, GIIb, GI.6, GII.1, GII.2, and GIIc. The median clinical severity of NoV AGE was 14 compared to 16 for RV AGE on a 20-point scale. *Conclusion*: NoVs were nearly as common as RVs as causative agents of severe AGE in children seen in hospital. After implementing universal RV vaccination, the importance of NoVs will still increase further.

Keywords Acute gastroenteritis · Child ·
Human calicivirus · Norovirus · Rotavirus

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Introduction

Noroviruses (NoVs) are major causative agents of acute gastroenteritis (AGE) in outbreaks in children and adults [1, 9–11]. In children, NoVs are also a common cause of seasonal AGE, as described in Finland in a study in 1993–1995 [15, 16] and later elsewhere [4, 9, 10, 24]. In hospital-based studies of seasonal AGE in children, the incidence of NoVs has been lower than that of RVs [9, 14, 15, 17, 26, 27], but particularly in community-based studies, NoVs have been the second most common, or sometimes even the most common causative agents of AGE in children [3, 5, 7, 10, 16–18]. Considering both frequency and severity, NoVs are the second most important cause of viral AGE in children [9, 16, 17, 26], and their importance will be further underscored following universal RV vaccination.

NoVs belong to human caliciviruses (HuCVs), which are divided into NoVs and sapoviruses (SaVs). Seasonal NoV AGE in children is most often caused by genogroup GII NoVs, and both GI and GII are seen in outbreaks. Since the mid 1990s, genotype GII.4 has emerged and become the predominant NoV type in outbreaks [23]. The emergence of GII.4 has also resulted in an overall increase of NoV outbreaks [14]. GII.4 is also the dominant strain detected in seasonal NoV AGE [23], which may have led to an increase of NoV AGE in children in general. The reason may be the greater virulence of GII.4 compared to other NoV genotypes [13, 28].

In this study, we investigated the occurrence and types of NoVs in children seen in Tampere University Hospital either as outpatients or admitted to a ward because of AGE. We also assessed the severity of NoV AGE in comparison to RV AGE.

Methods

Clinical methods

This prospective study was conducted in Tampere University Hospital in September 2006–August 2008. The hospital is the pediatric referral center for the Pirkanmaa Hospital District, a mainly urban area with a child population of about 79,000. The study protocol was approved by the Ethics Committee of Pirkanmaa Hospital District.

All children ≤15 years of age treated in the emergency room (ER), or admitted to the hospital ward for AGE, or caught nosocomial AGE while hospitalized for another reason in Tampere University Hospital, were eligible for enrolment. The diagnosis of AGE was made by a doctor in the hospital. The AGE was considered to be nosocomial if the symptoms began at least 24 h after hospitalization. Prior to enrolment, a parent or legal guardian was informed about the study and signed an informed consent form.

If the child had more than one ER visit or hospitalization during the study period, we deemed the AGE symptoms to belong to the same episode if there were fewer than seven symptom-free intervening days. Otherwise the visits were considered to represent two separate episodes.

At enrollment, the parents were interviewed about the child's AGE symptoms and treatment before the hospital visit, and their rotavirus vaccination status was ascertained. A questionnaire about the total duration of the symptoms after discharge was requested to be completed and returned after recovery. After discharge from the ER or the hospital ward, additional clinical information and possible laboratory test results of the AGE episode were collected from the medical records. A stool sample was collected while in the hospital. We failed to collect the sample if the child did not pass stools while in the hospital. If the parents did not return the questionnaire, the duration of symptoms at home remained unknown. Severity of the AGE episode was assessed using a 20-point score [19], in which ≥11 points is usually considered to be severe AGE. This score considers the following symptoms and features: fever, duration and intensity of diarrhea, duration and intensity of vomiting, degree of dehydration and treatment given, and need for hospitalization. If information on one or more of these items was missing, the case was excluded from the severity analysis. The statistical analyses were done using SPSS 15.0 with Mann–Whitney and Kruskal–Wallis tests.

Apart from the prospective surveillance, we also collected discharge information on all patients ≤15 years of age treated for AGE in Tampere University Hospital during the study period. For this purpose, all the ICD-10 diagnoses of groups A01–A09 were retrieved.

In the second follow-up season, an extensive waterborne AGE outbreak occurred in Nokia, a town close to Tampere. The outbreak was caused by massive contamination of drinking water by sewage water and caused extraordinary severe and mixed AGE [12, 21]. Because of the uncommon features associated with the AGE cases in this outbreak, we excluded the cases associated to this outbreak from this analysis; the cases have, however, been reported separately [19].

Laboratory methods

All stool samples were studied by reverse transcription (RT)-PCR for HuCVs and RVs. HuCVs were detected using a modified RT-PCR method introduced by Jiang and Farkas [2, 8]. These primers, localized in the RNA polymerase region, co-detect NoVs and SaVs: in this study, we describe the NoV findings. All PCR-positive amplicons were sequenced to confirm the PCR results and to determine the NoV genotype. Original genotypes for validation have been described earlier [2, 8]. We used NoV GI.1, GI.3, GI.4, GI.6, GII.1, GII.2, and GII.4 genotypes for PCR validation. In addition, we have later found other genotypes using this same PCR, such as GIIb, GII.7, GI.2, GII.9, GIIU, and GIId [6]. RV G types were determined by RT-PCR as described by Pang et al. [16] with the Taq polymerase replaced by GoTaq® polymerase (Promega, Madison, WI, USA). Both RT-PCR methods are highly sensitive detecting viruses and can be made from the smallest amount of sample as well as from diapers. Negative findings do not need confirming tests. If there was any uncertainty in the positive findings or sequencing, the tests were repeated.

The presence of RV antigen in stools was detected with ELISA using IDEIA® Rotavirus kit (Oxoid Ltd., UK) according to the manufacturer's protocol. The ELISA test is not as sensitive as the PCR techniques and cannot be performed from diapers.

Results

A total of 1,723 patients ≤15 years of age presented with AGE in the Tampere University Hospital during the study period. The number of patients recruited was 1,193, of whom 65 were excluded because of association with the waterborne AGE outbreak in Nokia [20], and thus, 1,128 (65% of the total cases of AGE) were included in this study. Among these, there were 45 (4%) children, who were known to have received at least one dose of either of the RV vaccines. Stool samples were obtained from 759 children (67% of included and 44% of all eligible)—341 in the first AGE season (September 2006–August 2007) and 418 in the second season (September 2007–August 2008).

The cases of AGE positive for HuCV and RV are shown in Table 1. In the first season, 116 out of 341 (34%) and, in the second season, 80 out of 418 (19%) AGE cases were positive for NoV, and in both seasons combined 196 of all 759 (26%). Of the NoV-positive cases, 24 (12%) were mixed infections with RV. In the first season, 4 (1%) out of 341 stools and, in the second season, 8 out of 418 (2%) stools were positive for SaV; of these, 2 were mixed infections with RV. There were no cases with both NoV and SaV or more than one type of NoV in the stools at the same time. No child had more than one episode of NoV AGE during the follow-up. RVs were present in 128 (38%) AGE cases in the first, and 260 (62%) in the second follow-up season, of which 26 (7%) were mixed infections with a NoV or SaV. RV was found in seven specimens from the children who were known to have received RV vaccines; of these, three were vaccine-type viruses from recent vaccination.

Genotype GII.4 was the predominant NoV genotype detected in both seasons. In the first season, 111 (96%) and, in the second season, 64 (80%) of the NoV-positive cases were of genotype GII.4 (Table 2).

The age range of the children treated because of NoV AGE was from 19 days to 13 years 8 months. The median age was 15 months, and the peak incidence was between 6 and 18 months of age. Altogether, 72% of children were ≤24 months of age. In the children treated because of SaV AGE, the age ranged from 6 months to 2 years 10 months, the median being 22 months (Fig. 1).

A clear seasonality was seen in NoV AGE in both years. The most active NoV period in the first season was from February to April 2007 and in the second season from January to April 2008 (Fig. 2). The SaV AGE cases were scattered with no obvious seasonality.

Of the AGE cases that had NoV as the only causative agent, 80 out of 172 (47%) were admitted to the hospital and 81 (47%) were treated as outpatients. The remaining 11 cases (6%) were nosocomially acquired. For comparison, of the 362 AGE cases with a RV as a single causative agent, 189 (52%) were admitted to the hospital, 161 (44%) were

Table 1 Causative agents of acute gastroenteritis in children seen in Tampere University Hospital in September 2006–August 2008

Virus	1st season (%)	2nd season (%)	Total (%)
Norovirus (NoV)	105 (31)	67 (16)	172 (23)
Sapovirus (SaV)	4 (<1)	6 (1)	10 (1)
Rotavirus (RV)	117 (34)	245 (59)	362 (48)
Mixed NoV + RV	11 (3)	13 (3)	24 (3)
Mixed SaV + RV	0 (0)	2 (<1)	2 (<1)
Other/undefined	104 (30)	85 (20)	189 (25)
Total	341 (100)	418 (100)	759 (100)

Table 2 Norovirus genotypes seen in acute gastroenteritis in children in two seasons (September 2006–August 2008) in Tampere University Hospital

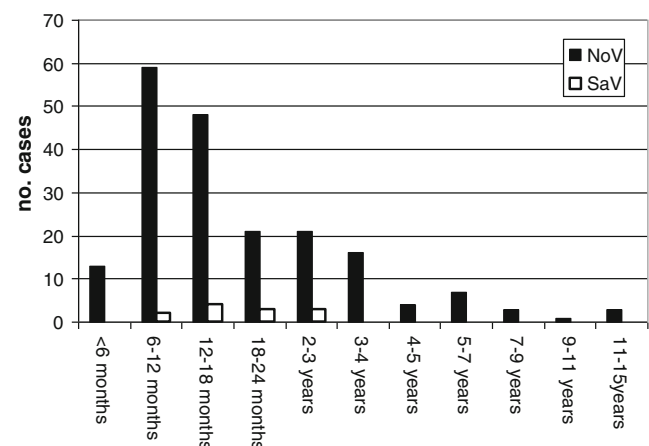| Genotype	1st season (%)	2nd season (%)	Total (%)
GII.4	111 (96)	64 (80)	175 (89)
GII.7	1 (<1)	4 (5)	5 (2)
GIIb	0 (0)	11 (14)	11 (6)
GII.1	2 (2)	0 (0)	2 (1)
GIIc	1 (<1)	0 (0)	1 (<1)
GI.6	1 (<1)	0 (0)	1 (<1)
GII.2	0 (0)	1 (1)	1 (<1)
Total	116 (100)	80 (100)	196 (100)

treated as outpatients, and 12 (3%) were nosocomial. Of the children admitted to the hospital because of AGE, NoV was the causative agent in 21% and RV in 50% of the cases.

Of the 369 cases from which the stool sample could not be obtained, 298 (81%) were treated as outpatients, 64 (17%) were admitted to a ward, and 7 (2%) were nosocomially acquired AGE. We enrolled 48 cases of nosocomially acquired AGE and obtained stool samples from 41 of these. In 11 (27%) of these, the causative agent was NoV, in 12 (29%) the causative agent was RV, and in 4 (10%) both NoV and RV were found. No SaVs were found in nosocomial AGE.

Clinical severity score could be calculated in 79 out of 171 (46%) of NoV-positive and RV-negative, and 196 out of 356 (55%) RV-positive and NoV-negative AGE cases. The severity scores of AGE cases caused by NoV ranged from 8 to 19, and cases caused by RV from 10 to 20 (Fig. 3). Median severity of AGE cases with a NoV as single causative agent was 14, and with a RV as a single causative agent, median was 16. The difference between the
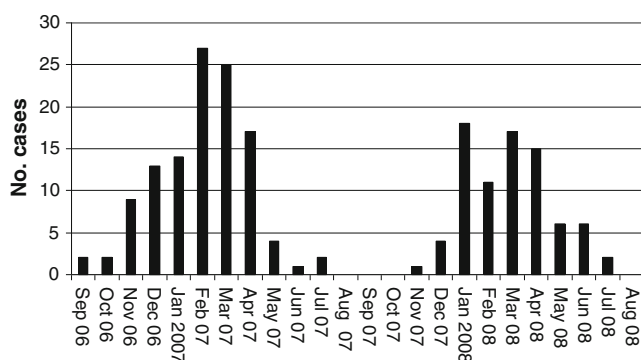

Fig. 1 Age distribution of children seen in Tampere University Hospital because of norovirus and sapovirus gastroenteritis in September 2006–August 2008

Fig. 2 Seasonality of the noroviruses seen in acute gastroenteritis in children (September 2006–August 2008) in Tampere University Hospital

severities of AGE caused by NoV and RV was statistically significant ($P<0.01$).

Discussion

This study confirmed both the prevalence and clinical importance of NoVs as the causative agent of seasonal AGE in children. At the hospital level, NoVs are, after RVs, the second most important causative agents of AGE in children admitted to a hospital or treated as outpatients. In some seasons, they may be as important as RVs, being as frequent as RV AGE, which was the situation in the first season, resulting in significantly severe cases of AGE. Overall, the 26% proportion of NoVs in the total material and the 21% proportion among children hospitalized with AGE appear higher than generally reported in the past. For example, in a review of published studies from 1990 to 2008, Patel et al. counted that the pooled proportion of

Fig. 3 Severity of clinical symptoms of acute gastroenteritis caused either by norovirus ($n=79$) or rotavirus ($n=196$) (September 2006–August 2008). Severity is assessed with a 20-point scale in which ≥11 points is considered as severe

NoVs was 11% [17]. We propose that the proportional increase may also reflect an absolute increase of NoV AGE in children, possibly due to greater virulence of the recently emerged genotype GII.4.

Of the 1,723 eligible children, 1,193 were recruited. The rest were lost mainly because of the ER being too busy to be able to recruit all the eligible cases. Sixty-five cases belonging to the AGE outbreak in Nokia were excluded from this analysis because of the extraordinary features of this outbreak leaving 1,128 cases in this study material. Furthermore, stool samples could be obtained only from 759 of the 1,128 enrolled children because the stools were collected only in the hospital. There were many children who spent only a few hours in the ER passing no stools during that time. A possibility of a bias in the AGE-causing agents resulting from these matters cannot be totally excluded, and the majority of stool samples are from the severe AGE cases. However, we could see no relation between the causative agent and passing stools in the ER. Even with these limitations, our study represents almost half (47%) of all the AGE cases seen in the hospital in children in the 2-year period.

NoV AGE showed seasonality with activity peaking in wintertime and only little NoV activity in the summer months. This confirms that NoVs are not involved only in outbreaks, but are also important causative agents in seasonal epidemic AGE. Like RVs, NoVs have winter seasonality, but in this study the seasons were distinct, with NoVs occurring earlier than RVs.

In the first follow-up year, from September 2006 to August 2007, RV activity was unusually low and associated with several RV G types. By contrast, RV activity in the second year from September 2007 to August 2008 was high and associated with the most common RV G-type G1P [8]. In the circumstances of low RV activity in the first season, the NoVs caused as many AGE hospitalizations as RVs, but when RV activity was high, the proportion of NoV AGE cases seen in the hospital was lower. Furthermore, the "virulent" NoV genotype GII.4 predominated particularly in the first season with a 96% share. It is tempting to speculate that simultaneous occurrence of the high NoV activity with the virulent GII.4 genotype and the low RV activity with multiple G types may be more than a coincidence. In any case, while RV G1P [8] re-emerged as the dominant RV type in the second season of follow-up, the NoV activity decreased, the proportion of GII.4 became less, and other NoV types appeared.

In general, NoV GII.4 has been the most common NoV strain detected in children, as well as in adults, this century. GII.4 activity varies from one season to another [22, 23, 28]. Such variation may be associated with mutations in the antigenic epitopes or other parts of the NoV genome [25, 28].
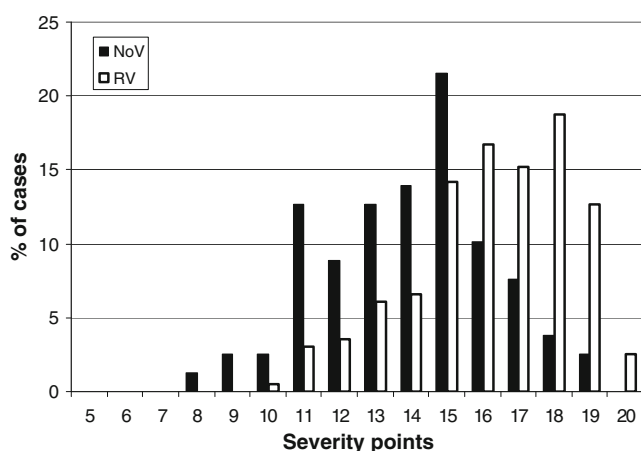
SaVs were not the point of primary interest in this study and are not discussed at length. SaV findings were single cases scattered throughout the follow-up: 12 cases were seen, of which 2 (17%) were mixed AGE cases with RV.

We supposedly did not obtain information about all the nosocomially acquired AGE in the hospital in the study period. If the child is hospitalized for another reason and gets AGE symptoms, he/she is usually discharged as soon as possible, and unfortunately, often no ICD-10 diagnosis of AGE is recorded. Nosocomial AGE is not discussed in-depth here because its real incidence is probably higher than what is seen in this study.

We used the 20-point severity scale to assess the severity of those AGE episodes which were not associated with the Nokia AGE outbreak of November–December 2007. The severity of NoV cases seen in the hospital was somewhat lower than that of RV cases (Fig. 3). However, the median severities of the AGE cases caused either by NoV, RV, or both were over 11, which is considered severe. If we assess the severity of AGE using only the hospitalization rate among the children, NoV AGE cases seem almost as severe as RV cases: of the NoV AGE cases, 47% were hospitalized and 47% were treated as outpatients; of the RV AGE cases, 54% were hospitalized and 42% were treated as outpatients. The number of non-GII.4 types seen was small and did not permit statistical comparison of clinical severity between GII.4 and other NoV genotypes. Median severity of the AGE caused by GII.4 NoV was 14 ($n=71$) with a range from 8 to 19.

Presumably, the overall high severity of the AGE cases in our study was reflected in the hospital-based study design and in the fact that we did not obtain stool samples for analysis from many of the cases not needing hospitalization. However, the NoV AGE cases seen in this study were unexpectedly severe, even in a hospital setting. This may be a sign of the proposed greater severity of genotype GII.4 NoVs compared to the other genotypes. The omission of such cases may influence the assessment of mean clinical severity of AGE associated with NoV or other gastroenteritis viruses.

There are only a few prospective follow-up studies on both endemic circulating NoV genotypes and severity of endemic NoV AGE in children. The results of this survey also serve as a baseline to subsequent studies of AGE in children to be conducted after the introduction of universal RV vaccination in Finland in September 2009. It is expected that the proportional role of NoV will increase along with a decrease in RVs. It will be of particular interest to see if the absolute numbers of NoV AGE also increase in the future.

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ORIGINAL ARTICLE

Major reduction of rotavirus, but not norovirus, gastroenteritis in children seen in hospital after the introduction of RotaTeq vaccine into the National Immunization Programme in Finland

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Abstract Universal rotavirus (RV) vaccination is expected to reduce hospitalizations for acute gastroenteritis (GE) of children by eliminating most of severe RVGE, but it does not have any effect on norovirus (NV), the second most common causative agent of GE in children. After the introduction of the RV vaccine into the National Immunization Programme (NIP) of Finland in 2009, we conducted a prospective 2-year survey of GE in children seen in Tampere University Hospital either as outpatients or inpatients and compared the results with a similar 2-year survey conducted prior to NIP in the years 2006–2008. Compared with the pre-NIP 2-year period, in 2009–2011, hospitalizations for RVGE were reduced by 76 % and outpatient clinic visits were reduced by 81 %. NVGE showed a slight decreasing trend and accounted for 34 % of all cases of GE seen in hospital in pursuance of RVGE having decreased to 26 % (down from 52 %). In cases admitted to the hospital ward, RV accounted for 28 % and NV accounted for 37 %.The impact of RV vaccination was reflected as a 57 % decrease in all hospital admissions and 62 % decrease in all outpatient clinic visits for GE of any cause. *Conclusion*: RV vaccination in NIP has led to a major reduction of hospital admissions and clinic visits due to RVGE, but has had no effect on NVGE. After 2 years of NIP, NV has become the leading cause of acute GE in children seen in hospital.

Introduction

Rotaviruses (RVs) and noroviruses (NVs) are the two most common causative agents of acute gastroenteritis (GE) in children <5 years of age in Finland [17, 27]. In all resource-rich countries combined, RVs and NVs cause annually an estimated 1,500,000 episodes of GE requiring a hospital visit [22].

A major reduction in severe RVGE has already happened or is expected to happen in countries with extensive use of vaccines against RV [21]. As a consequence, while overall severe GE is expected to decrease, the proportional role of NVs in childhood GE is likely to increase for NV to become the leading cause of GE requiring in-hospital admission [10, 12].

In Finland, RV vaccines were licensed in 2006, and the vaccination coverage rose from 0 to about 30 % between 2006 and 2008. In this pre-National Immunization Programme (NIP) period, we conducted a 2-year prospective study on RVs and NVs as causative agents of GE in children and found RVs to account for 52 % and NVs to account for 25 % of GE seen in hospital [27].

In the season 2008–2009, no prospective surveillance was ongoing. RV vaccination with exclusive use of bovine–human reassortant RV vaccine RotaTeq® (RV5, Merck & Co. Inc.; in Europe, Sanofi Pasteur MSD) was included into the Finnish NIP on 1 September 2009. The coverage of vaccination rose quickly to over 90 % and reached a level 95–97 %, similar to other vaccines in NIP (source: National Institute for Health and Welfare [THL], Finland). The present study, following the same methodology as the pre-NIP

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study, was started at the same time with the introduction of RotaTeq into the NIP and continued for a 2-year period 2009–2011. This enabled us to compare the absolute numbers and proportions of RVs and NVs in acute GE (AGE) seen in the hospital before and after universal RV vaccination.

Materials and methods

Clinical methods

The prospective study was conducted at Tampere University Hospital from September 2009 to August 2011. The hospital is the pediatric referral center for the Pirkanmaa Hospital District, a mainly urban area with a birth cohort of approximately 6,000 children. The study was approved by the Ethics Committee of Pirkanmaa Hospital District.

All children under 16 years of age seen in the emergency room (ER) or admitted to a pediatric ward with AGE were eligible for enrolment. Prior to enrolment, a parent or legal guardian provided a written informed consent for participation.

Parents were interviewed about their child's symptoms before the hospital visit and about their child's RV vaccination. A study nurse confirmed the vaccination status from the records of the respective well baby clinic. A stool specimen was collected during the hospital visit in the ER or at the hospital ward.

If the child had required more than one ER visit or hospitalization due to AGE during the study period and if there were more than seven symptom-free days between them, they were considered as two separate episodes.

Laboratory methods

All stool specimens were tested for the presence of RV and human caliciviruses (including NVs and sapoviruses [SaVs]) using a reverse transcription polymerase chain reaction (RT-PCR) method, as described previously [8, 15, 25–27].

After the detection of RV, the G and P genotypes were determined by nucleotide sequencing of the gene segments encoding for the VP7 and VP4 antigens. The gene segment encoding for VP6 protein was also sequenced to determine the presence of vaccine-derived virus [13].

After the detection of human caliciviruses, RT-PCR typing targeting region C at the beginning of the NV capsid region in open reading frame 2 was done with primers JV21, JV24, and JV24mod [3, 35]. The NV genotypes were defined as the polymerase region A/capsid region C genotype.

RV-positive and NV-positive PCR products were sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA).

Nucleotide sequences read from the chromatograms were aligned to published sequences from GenBank (http://

www.ncbi.nlm.nih.gov/genbank/) and from the Food-borne Viruses in Europe network (http://www.rivm.nl; National Institute of Public Health and the Environment, The Netherlands).

Statistical analyses

Statistical analyses were performed using the Mann–Whitney U test to compare the age distributions of RVGE and using the chi-square test to calculate the reductions in RVGE between the two study years and two reference years; both tests were performed in SPSS, version 20.0 (SPSS). All tests were two-tailed and a p value <0.05was considered to be statistically significant.

Reference years

The results were compared to reference years of 2006–2007 and 2007–2008 (both seasons from September to August), during which prospective surveillance for RV AGE had been conducted in the same setting using the same methodology [27, 29]. In the second study year in late 2007, an extensive waterborne AGE outbreak occurred in the town of Nokia caused by massive contamination of drinking water by sewage water [27, 28]. We excluded 65 patients associated with this outbreak from the comparative NV analysis to better reflect a normal situation of endemic NVGE [7, 16, 20, 36]. For the RV analysis, those patients were not excluded.

Results

In the 2-year period from 1 September 2009 through 31 August 2011, a total of 495 patients were recruited for the study at Tampere University Hospital. Stool samples were obtained from 330 children (66 % of those recruited)—160 in the first season (September 2009–August 2010) and 170 in the second season (September 2010–August 2011). Of these 330 children, 144 (44 %) were treated in the ER and 186 (56 %) were admitted to a pediatric ward.

For comparison, in the 2-year period of 2006–2008, 1,193 patients were recruited and stool samples were obtained from 809 (68 % of those recruited) children; of whom 434 (54 %) were hospitalized and 375 (46 %) were treated as outpatients.

Rotavirus gastroenteritis

In 2009–2011, of the 330 cases with AGE with a stool specimen, 86 (26 %) were found to have a wild-type RV in stools; 34 of those were treated as outpatients and 52 were hospitalized. Compared to reference years 2006–2007 and

2007–2008 (combined), this means an 81 % reduction of RV AGE in the outpatient clinic (34 vs. 177 cases) and a 76 % reduction in hospital ward admissions (52 vs. 219 cases) (Fig. 1).

The total reduction of all RVGE cases was 80 % during the study years and the proportion of RVGE cases of all AGE cases was decreased from 52 % (421 cases) in the two reference years to 26 % (86 cases) in the two study years combined. The total reduction was statistically significant with $p<0.001$.

RV was found in 43 cases each (27 and 25 %, respectively) of 160 and 170 stool samples obtained in the first and the second season after NIP, respectively. In the first RV epidemic season, the majority of RV-positive AGE cases were seen relatively late between March 2010 and May 2010, whereas in the second season, the most active months were between January 2011 and March 2011 (Fig. 1).

The age distribution of the children with RVGE was from 7 months to 14 years 6 months. The age distribution of RVGE patients shifted towards older children each year ($p<0.001$). In 2006–2008, the median age was 19 months, while in 2009–2010, it was 24 months, and in 2010–2011, it was 36 months. Still, the proportion of RVGE cases decreased in every age group, even among children too old for vaccination. The reduction in the age groups eligible for vaccination (patients <1 year of age in 2009–2010 and patients <2 years of age in 2010–2011) was 91 % (16 vs. 178 patients), and in children too old to be vaccinated in NIP, it was 72 % (70 vs. 243 patients). The age distribution of RV-positive cases during 2006–2008 and 2009–2011 are shown in Fig. 2.

The predominant RV types in the two seasons 2009 to 2010 and 2010 to 2011, combined, were G1P[8] ($n=38$, 44 %) and G4P[8] ($n=30$, 35 %). In the first season, genotype G4P[8] ($n=18$, 42 %) was slightly more common than G1P[8] ($n=15$, 35 %), but in the second season, genotype G1P[8] was more predominant with 53 % ($n=23$) over G4P[8] ($n=13$, 30 %). Other common RV genotypes G2P[4], G3P[8], and G9P[8] were all seen to a lesser extent ($n=9$, $n=1$, and $n=6$, respectively, counted from all RV-positive AGE cases in the two seasons combined). In two cases, more than one RV type was found in stools simultaneously: in one case, G1P[8] with G3P[8] and, in the other, G3P[8] with G9P[8]. Other than the predominance of G9P[8] genotype in the season of 2006–2007, no great changes in the genotype distribution were observed during the study years compared to the reference years (data not shown).

Among the 86 wild-type RV-positive cases, there were 4 children who had received at least 1 dose of RotaTeq® and 1 child who had received Rotarix™ before the introduction of NIP. Three of those who had received RotaTeq® were fully vaccinated (two were detected with G4P[8] in the stools and one was detected with G9P[8] in the stools) and one had received only one dose and was detected with G4P[8] RV. The child who had received Rotarix™ was also fully vaccinated with two doses and was detected with the G4P[8] genotype. Two of the four breakthrough cases, 9- and 10-month-old fully vaccinated boys (RotaTeq®), were admitted to the pediatric ward and the other two were seen in the ER only.

We identified three cases of GE in young infants shedding a human–bovine double reassortant G1P[8] vaccine virus. This human–bovine double reassortant was also detected from one patient infected concomitantly with NV. Furthermore, one patient was detected with RotaTeq® vaccine virus G6P[8] and 16 patients were detected to shed the original vaccine virus G1P[5] or just the VP7 G1 part of it separately or detected with several VP4 proteins. No patients were detected with Rotarix™ vaccine strain after 2006–2008. The vaccine-associated cases have been reported separately [13].

Fig. 1 Timing of the RV seasons between September 2006 and August 2008 and September 2009 and August 2011 and treatment needed in the 507 children seen at Tampere University Hospital because of acute RVGE
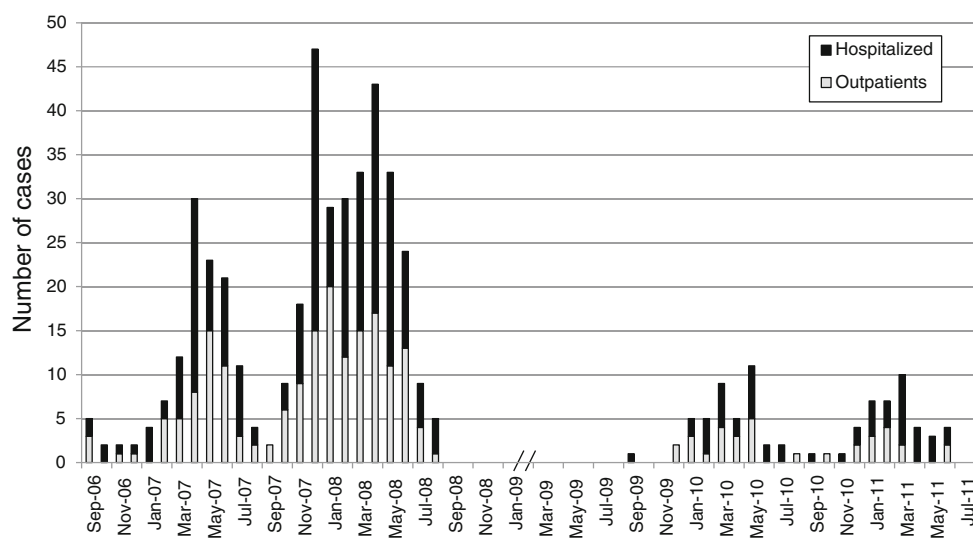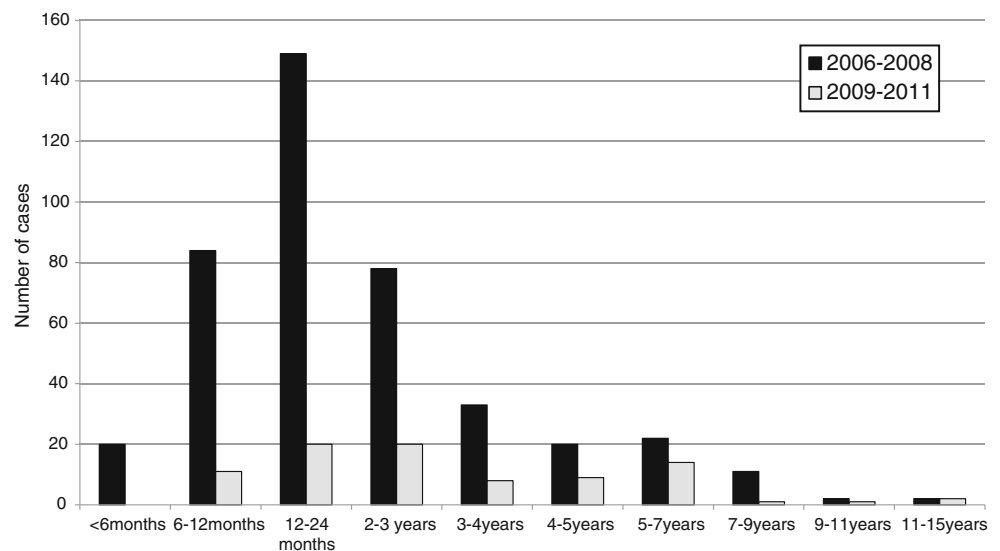
Norovirus gastroenteritis

Of all 330 cases of GE in 2009–2011, 111 (33.6 %) were
NV-positive. In the first year, NV was found in 52 (33 %) of
160 stool samples and, in the second season, in 59 (35 %) of
170 stool samples (Fig. 3). SaV was found in a total of 23
(7.0 %) cases, 13 (8.1 % of 160) of these in the first season
and 10 (5.9 % of 170) in the second season.

Of the NV-positive cases, only one was a mixed infection
with RV (more specifically with G2P[6]). Three of 23
(13 %) SaV-positive cases were mixed infections with
RVs. There were no cases with NV and SaV in the stools
at the same time. The reduction of GE positive for NV, SaV,
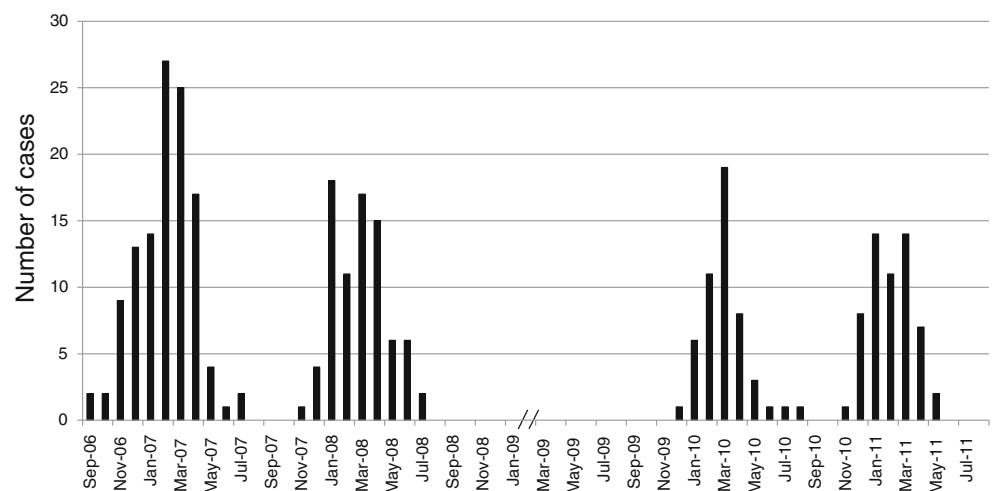and RV is shown in Fig. 4.

In the reference years 2006–2008, there were 196 cases
(excluding the outbreak mentioned in the "Materials and
methods" section) of NVGE as compared with 111 cases
in the study years 2009–2011. Of the 111 NV-positive cases,
69 (62 %) were admitted to the hospital and 42 (38 %) were
treated as outpatients. Even though the absolute number of

NV-positive cases decreased slightly (196 vs. 111 cases), the
proportion of NVGE of all AGE increased (26 vs. 33.6 %)
from the reference years. Moreover, the proportion of NV-
positive cases that were admitted to the hospital increased
from 47 % (92) in the reference years to 62 % (69) in the
study years (Fig. 4).

Compared to the reference years, the proportion and the
absolute number of SaV-positive GE increased from 1.6 % (12
cases) to 7.0 % (23 cases). A clear seasonality was seen in the
NVGE both in the study years and in the reference years
(Fig. 3). The most active months when the majority of NV-
positive cases were seen were between January and April in
each year.

Of all 111 NV-positive cases, 108 (97 %) were gen-
ogroup GII strains and 72 (65 %) were genotype GII.4 (37
(71 % of 52 cases) and 35 (59 % of 59 cases) in the first and
the second study years, respectively). In the reference years,
genotype GII.4 was even more common with 89 % propor-
tion (175 of 196 cases). The other genotypes detected were
GII.b (14 %, $n=15$), GII.7 (13 %, $n=14$), GII.g (5 %, $n=6$),

Fig. 4 Viruses detected in the stools of children admitted to the hospital for AGE in 2006–2008 and 2009–2011
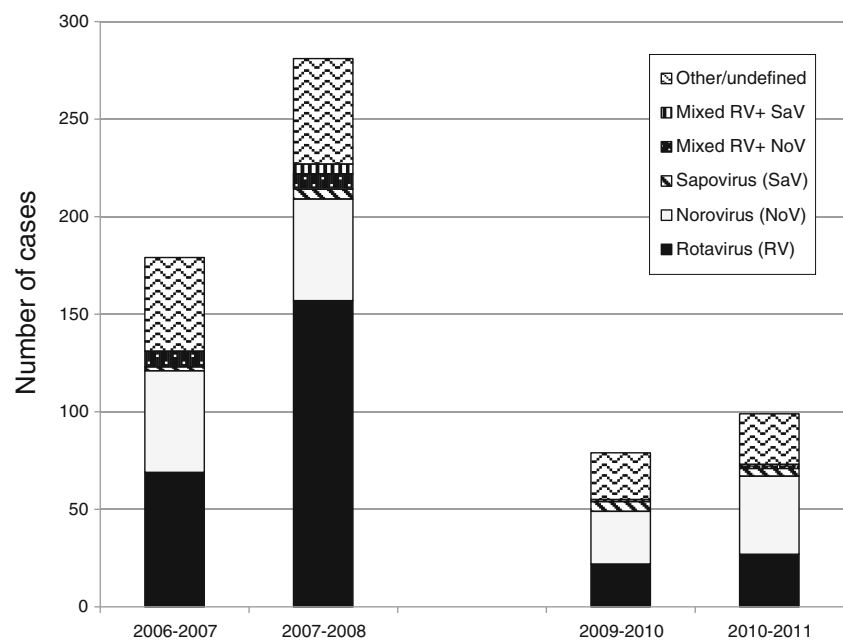
GI.4 (2 %, *n*=2), GI.3 (1 %, *n*=1), and GII.e (1 %, *n*=1). Additional genotypes detected in the reference years included GII.1, GII.c, GI.6, and GII.2, all of which accounted for <1 % each and none of which were detected in the study years 2009–2011.

The age distribution of NV-positive children was similar between the study years and the reference years. The age range was from 7 days to 15 years 7 months (19 days to 13 years 8 months in reference years), with a median age of 12 months (15 months in reference years). Eighty-four of 111 cases (76 %) of children were under 24 months of age, and 41 % was under 12 months of age.

Gastroenteritis with neither RV nor NV

In the study years 2009–2011, of the 330 cases of AGE, 222 (67 %) were positive for RV, NV, or SaV alone or as a mixed infection, whereas in the reference years 2006–2008, 603 (76 %) were positive for RV, NV, or SaV alone or as a mixed infection. The absolute number of GE cases due to other pathogens decreased from 191 cases in the reference years to 108 cases in the study years. Of 108 cases, 53 % (57 cases) detected in the two study years was admitted to a pediatric ward and 47 % (51 cases) was treated as outpatients. RV, NV, or SaV could be detected in the stools in 70 % of all 186 cases admitted to a pediatric ward due to GE and, conversely, 30 % was negative for these viruses (Fig. 4). The absolute number of children who were admitted due to GE and were negative for RV, NV, or SaV decreased from 101 cases in the reference years to 57 cases in the study years. Even though a systematic search for other GE viruses was not performed, some of these patients were found to have other viral agents such as human bocavirus,

adenovirus, astrovirus, and coronavirus in their stools [30, 31]. In the emergency department, 35 % (51 of 144 cases) of the children seen for GE symptoms were negative for RV, NV, or SaV. All hospital admissions due to all AGE decreased by 57 % from 434 cases in the reference years to 186 cases in the study years, and the proportion of outpatient clinic visits decreased by 62 % from 375 to 144 cases.

Discussion

In this study, we examined the impact of the National RV Immunization Programme (NIP) on hospitalizations and outpatient clinic visits due to GE in one hospital. The coverage population of the Tampere University Hospital is about one tenth of Finland, and the results may be generalized for the whole country.

We detected a significant reduction in outpatient AGE visits and hospital admissions due to RV (81 and 76 %, respectively) in the 2-year post-NIP period in the entire children population. Similar reductions with the exclusive use of RotaTeq have been observed previously from the USA [32, 33, 37] and from countries using both two available RV vaccines [2, 4, 23].

We did not observe further reduction in RVGE between first (2009–2010) and second (2010–2011) seasons. This might be because the second season post-NIP (2010–2011) might have been a strong epidemic season of RV, resulting in more RV infection pressure; just like in the two reference years, season 2007–2008 was a high epidemic season compared to 2006–2007.

Our study supports the evidence of herd protection in children too old to be vaccinated that have been observed in three studies from the USA after the widespread use of RV

vaccines [5, 9, 37]. We observed that the reduction in RVGE cases was statistically significant in every age group. The decrease in hospitalizations in children too old to be vaccinated was 72 %, similar to the findings from the USA (70–79 %) [5, 9, 37]. We found no cases of wild-type RVGE in children <6 months of age. In contrast, in the USA, no reduction in infants <3 months of age, who had been too young to be vaccinated, was observed [5, 9, 37]. Additionally, we observed that the median age distribution of RVGE cases had shifted toward older children.

The high level of herd protection in our study probably resulted from high vaccine coverage. In Austria, no evidence of herd protection was found with vaccine coverage of 57 % in 2007 (RotaTeq) [2]. The reason for herd protection is probably interruption of RV transmission among all children. Exposure of unvaccinated children to vaccine virus shed in the stools of vaccinated infants is possible but unlikely to explain herd protection. Such transmission of vaccine-acquired virus resulting to a symptomatic RVGE has been reported in several countries [24]. In our study, we detected shedding of vaccine virus in a number of children, but all were recently vaccinated and none was unvaccinated.

After the introduction of the RV vaccine into the vaccination program, several studies have detected unusual non-vaccine-included RV strains, such as G8 or G12, or changes in the genotype distribution [14]. However, none of these changes were observed in our study.

In addition, we observed that the impact of RV vaccination was reflected as decreased hospital admissions and outpatient clinic visits for GE of any cause. Compared to the pre-NIP period, there was a 57 % reduction in cases admitted to the hospital ward for all GE. The reduction was higher than the reduction rates observed in previous studies from the USA (29–52 %) [5, 6, 37].

In addition, we observed a reduction of 62 % in all outpatient clinic visits for GE of any cause. Interestingly, such a reduction in all outpatient clinic visits has not been reported from countries where the protective effect of RV vaccination in unvaccinated children has been observed [10, 12].

The important role of NV as a causative agent of endemic (not outbreak-associated) GE in children was first discovered in Finland in connection with an efficacy trial of RotaShield vaccine [18]. In the same study, it was observed that RV vaccine (RotaShield) did not have any effect on NVGE. In that sense, the present findings on the impact of universal RV vaccination on NVGE are (only) confirmatory, and we conclude that the RotaTeq vaccination program does not reduce NVGE. The slight decrease observed in the study vs. reference years may well be explained by natural annual variation. In a decade, there has been considerable year to year variation of NVGE, although the winter epidemic has occurred every year [26].

In reverse, other viruses and notably NVs could theoretically replace RV after its elimination by universal vaccination and fill the available niche as a major causative agent of AGE in children. Our results strongly suggest that such a replacement is not happening. Overall hospitalizations have been reduced according to the share of RV, and NV has become a leading cause of GE only in relative terms, without any increase in absolute numbers.

The reduction of all hospitalizations (57 %) and outpatient clinic visits (62 %) due to GE is well in line with what was observed in the prelicensure efficacy trial (REST) of the RotaTeq vaccine in Finland. RotaTeq reduced all-cause GE requiring medical intervention by 65 % over a period of 3.1 years [34]. To compare the numbers, it should be noted that the present population-based study also includes children who were eligible for vaccination but did not receive it (initially about 10 %, decreasing to 5 % over time).

The present study focused on NV and was not intended as a full etiological examination of GE. Hospitalizations due to GE not associated with RV or NV seemed to decrease somewhat in comparison with the reference years. This observation should be viewed with caution, and a detailed etiological study of GE viruses should be performed before conclusions. However, even though the RV vaccine had no effect on NVGE, it is nevertheless possible that RV vaccination might have a "nonspecific" effect on non-RV-associated GE. Some suggestive evidence of RV vaccine (RotaShield) effect on adenovirus GE was seen in the study in the 1990s [19].

The new leading role of NV as the main causative agent of AGE in children supports the concept of developing an NV vaccine for use in children [11]. Such a vaccine is foreseen and being developed [1].

Conclusion

RV in the NIP of Finland had an immediate and major impact on RVGE cases seen in hospital, i.e., severe RVGE. The age distribution of children with RVGE has shifted upwards at the same time as a statistically significant decrease in every age group was observed as an evidence of herd protection. The impact of RV vaccination was reflected in a decrease of all hospital admissions and outpatient clinic visits for GE, while NV has become the leading cause of AGE in children seen in hospital.

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