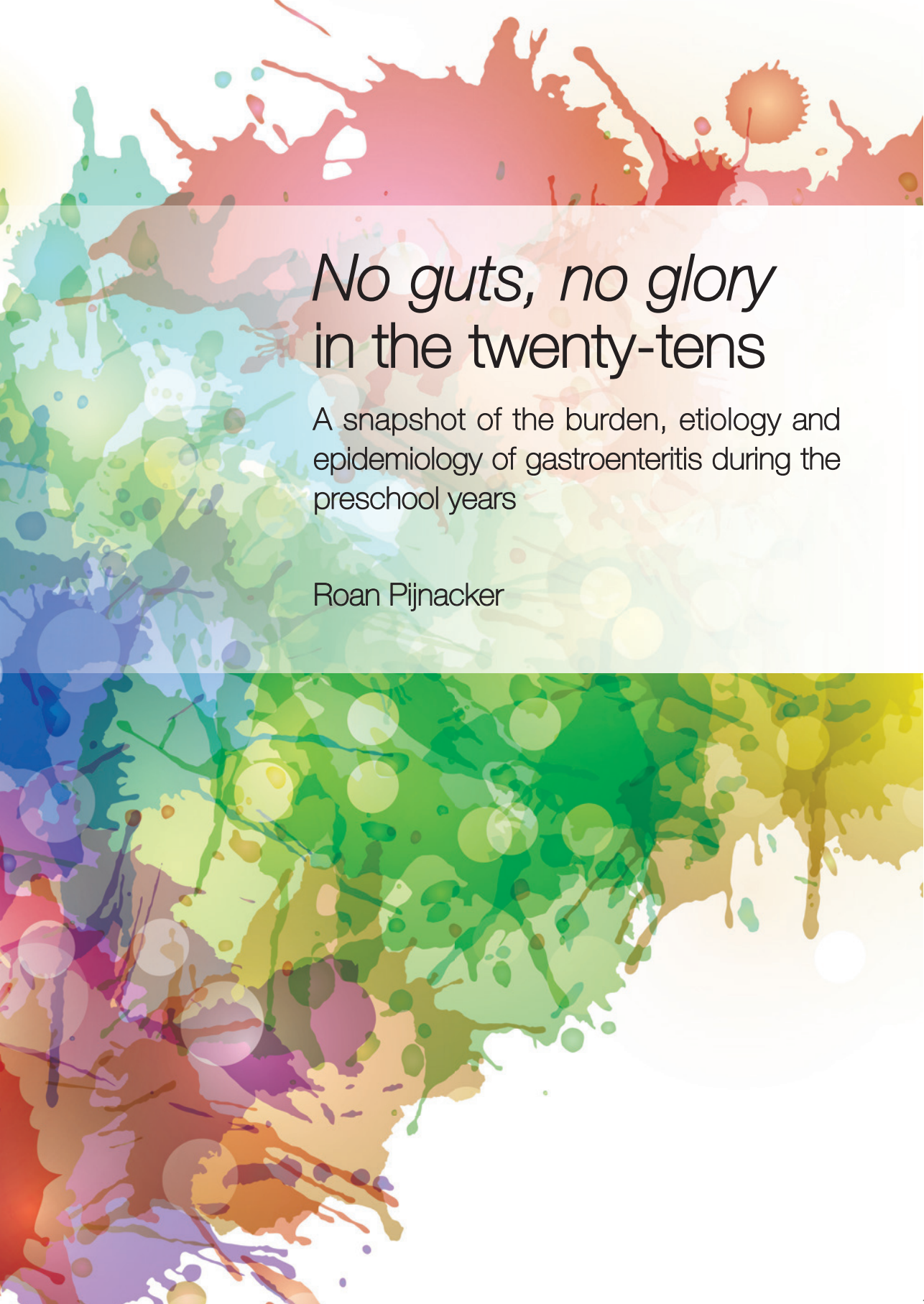

No guts, no glory in the twenty-tens

A snapshot of the burden, etiology and epidemiology of gastroenteritis during the preschool years

Roan Pijnacker

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Thesis Utrecht University

ISBN: 978-94-6375-406-4

Layout and design: Elisa Calamita, persoonlijkproefschrift.nl
Printing: Ridderprint BV | www.ridderprint.nl

The publication of this thesis was financially supported by the National Institute for Public Health and the Environment.

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A snapshot of the burden, etiology and epidemiology
of gastroenteritis during the preschool years

No guts, no glory in het afgelopen decennium

Een momentopname van de ziektelast, etiologie en epidemiologie van
gastro-enteritis tijdens de voorschoolse jaren

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van
de rector magnificus, prof. dr. H.R.B.M. Kummeling, ingevolge het besluit van
het college voor promoties in het openbaar te verdedigen op dinsdag 4 juni 2019
des ochtends te 10.30 uur

door

Roan Pijnacker

geboren op 13 maart 1989 te Woerden

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Contents

CHAPTER 1

General introduction

What is gastroenteritis?

In adults, about 8 to 9 liters of fluid material pass through the gastrointestinal tract daily (1, 2). These consist of oral intake and secretions of the upper intestine, saliva, stomach and other organs (3). The intestines absorb most of this material and only 100–200 ml is excreted with the feces (1, 3). Infection with enteropathogens or opportunistic microorganisms can disrupt the balance of secretion and peristalsis, leading to gastroenteritis (GE), a condition commonly defined as three or more loose stools within 24 hours and/or any vomiting event other than regurgitation, vomiting due to motion sickness/vertigo, traumatic event, nauseous event, or drug/alcohol abuse (2, 4). GE may be accompanied by nausea, abdominal cramps, fatigue, headache and fever. Although GE is most often caused by infectious agents, it can also have non-infectious causes such as medicines, food allergies/intolerances or chronic gastrointestinal diseases, such as gastrointestinal cancer, ulcerative colitis, Crohn's disease, etc. Fluid loss due to diarrhea and vomiting can lead to dehydration, which is particularly dangerous in infants and the elderly. Serious and sometimes long-term (extra-intestinal) complications may also occur, such as hemolytic-uremic syndrome (HUS) following infection with *Shiga* toxin-producing *Escherichia coli* (STEC), Guillain-Barré syndrome after *Campylobacter* infection, or Irritable Bowel Syndrome (IBS) following infection with *Campylobacter*, *Salmonella*, *Shigella*, *Clostridium difficile*, *E. coli*, *Yersinia* or *Entamoeba histolytica* (5-7).

No guts, no glory

Diarrheal illnesses were described by Hippocrates (460 – 370 B.C.) as an "abundant liquid stool at short intervals" (8). For hundreds of years, the causes of diarrhea were misunderstood and empirical treatment was often based on poor knowledge of the disease (9). During the 18th century, diarrhea was one of the most important causes of morbidity and mortality among troops engaged in battle, causing large epidemics among armed forces where hygienic and dietary conditions were generally poor (9, 10). In some battles, such as those during the Napoleonic Wars (1803 – 1815), diarrhea caused more deaths than war injuries (11). As a result, the motto "no guts, no glory" became a recognized code among war troops, and 'guts' became a synonym of bravery (12). It also became more widely appreciated that diarrheal disease prevention was essential in the success of military campaigns (11). During the course of the 20th century, based on the discovery of microorganisms as the cause of many diseases, public health actions were taken to control infectious diseases (13). The discovery of antibiotics in 1940, improvements in sanitation and hygiene,

mainly from the 1930s through the 1950s, and use of oral rehydration therapy for patients with diarrhea led to a significant reduction in morbidity and mortality of diarrheal disease in modern industrialized countries.

Burden of gastroenteritis

GE remains a leading cause of morbidity and mortality worldwide, accounting for an estimated 1.6 million deaths in 2016 (14, 15). More than a quarter (27%) of those deaths occurs in children younger than 5 years and the majority (89%) in South Asia and sub-Saharan Africa. In high-income countries like the Netherlands, the disease is rarely life threatening owing to universal access to high-quality healthcare, safe water and sanitation. Nonetheless, GE still causes a significant burden in terms of societal costs because of its high frequency of occurrence, particularly in vulnerable groups (e.g. children). The total annual costs of GE (including all age groups) was estimated to be €611-695 million in the Netherlands in 2009, corresponding to €133-151 per GE episode (16). Moreover, although the disease is usually mild and self-limiting, it may sometimes be grave in children, the elderly and in those immunocompromised.

The overall incidence of GE in the Netherlands was estimated at 283 per 1,000 person-years in a prospective population-based study in 1999, corresponding to 4.5 million GE episodes annually (17). The incidence was highest in children younger than 5 years of age, with four out of five children (832 per 1,000 children) experiencing an episode of GE once a year (17, 18). It was estimated that 1 in 20 cases with GE visited the general practitioner (GP) due to the GE complaints (19). Ten years later, in 2009, a 1-year retrospective survey estimated the community incidence of GE in the Netherlands to be more than three-fold higher (964 per 1,000 person-years), corresponding to 15.9 million episodes yearly, and 1 in 13 cases visiting the GP (20). Close to three episodes per year occurred in children below 5 years (2,894 per 1,000 person-years). The much higher estimates in 2009 were thought to be due to the retrospective nature of the study, which is prone to overestimation. Indeed, in the UK, they also found the incidence of GE to be 2.8 times higher in a retrospective survey than a parallel prospective study conducted in the same population (21). This was thought to be due to 'telescoping' bias, which refers to persons remembering their GE episode to be more recent than it actually is, and selection bias of persons who recently experienced GE. Based on national Dutch Hospital Discharge Data, between 6,000 and 10,000 children below five years were hospitalized for GE yearly during 2006 – 2013 (22).

In the Netherlands, around 50% of children attend a day-care center (DCC) before they reach elementary school age at 4 years. A study conducted in 2010–2013 estimated that the costs per GE episode (€229) were substantially higher for DCC-attending children than for home-cared children (€122) (18). This difference was mainly due to productivity losses of parents of DCC-attending children that had to care for their ill children. Children attending a DCC had a slightly but significantly higher rate of GE (1,251 per 1,000 child-years) compared with children cared for at home (893 per 1,000 child-years) (18). Additionally, higher GE hospitalization rates were found in a Danish cohort study of children attending DCC until one year of cumulative DCC attendance as compared to children cared for at home (23). After one year of DCC attendance, the risk of GE was lower compared to home-cared children, but such 'protective effect' was no longer observable during the elementary school years.

Each year, the Dutch Ministry of Health, Welfare and Sports (VWS) requests the Dutch National Institute for Public Health and the Environment (RIVM) to estimate the disease burden and cost-of-illness of 14 food-related pathogens in the Netherlands (24). Ten of these pathogens cause GE (i.e. *Campylobacter*, STEC O157; *Salmonella*, *Bacillus cereus*, *C. perfringens*, *Staphylococcus aureus*, norovirus, rotavirus, *Cryptosporidium*, *Giardia*), and the other four pathogens (i.e. *Listeria monocytogenes*, *Toxoplasma gondii*, hepatitis-A virus and hepatitis-E virus) cause other diseases. The disease burden is expressed in Disability Adjusted Life Years (DALYs), which is a metric integrating morbidity and mortality in one unit that allows for comparison of the burden of different (infectious) diseases. In 2017, the largest disease burden at population level was caused by *Campylobacter* (3,100 DALYs) followed by norovirus (1,600 DALYs). The highest annual costs were due to norovirus (€90 million) and rotavirus (€61 million).

Etiology of gastroenteritis

A wide spectrum of bacteria, viruses and protozoa may cause GE. Viruses are generally the most common GE-causing agents during the coldest months of the year, bacteria during the summer, and protozoa have a less pronounced seasonal pattern (25, 26). In high-income countries, viruses are the most common cause of GE in children, particularly rotavirus and norovirus (27-29). Other important causes of GE are astrovirus, adenovirus, sapovirus, *Shigella*, *Campylobacter*, pathogenic *E. coli*, *Yersinia*, *Cryptosporidium*, *Giardia*, and *E. histolytica* (29-31). The evidence that *Dientamoeba fragilis* causes GE is inconclusive (32). Since the

licensing of two rotavirus vaccines (Rotarix and Rotateq) for use in Europe in 2006, the relative contribution of rotavirus to childhood GE has dropped substantially in many countries (28, 33-37). European countries that introduced universal rotavirus vaccination observed reductions in rotavirus hospitalizations between 65% and 84% (33). This led to norovirus becoming the leading cause of medically attended GE in countries with high vaccination coverage for rotavirus (28). In the Netherlands, rotavirus will be implemented as part of the immunization programme in 2019, but only for children with medical risk conditions predisposing to severe or complicated rotavirus GE, including prematurity, low birth weight, and severe congenital pathology (38).

The distribution of enteropathogens (i.e. potential causative agents of GE) in children below 5 years of age can be different between GE cases in the community and those seeking healthcare because some pathogens are more likely to cause severe disease than others, which influences healthcare-seeking behavior (see Table 1) (19, 39, 40). For example, a study in children hospitalized for GE in the Netherlands found much higher prevalence rates of rotavirus (54%) than a study among children presenting at the GP with GE (16%), as well GE cases in the community (10%) (17, 41, 42). This reflects rotavirus as the most common cause of severe diarrhea in young children (31). Similar findings were reported in the 2005 REVEAL study conducted in Belgium, France, Germany, Italy, Spain, and Sweden (43). They found that prior to the introduction of rotavirus vaccination in these countries, rotavirus in children under 5 years of age was responsible for between 53% and 69% of cases presenting in hospitals, between 35% and 63% in emergency departments, and between 8% and 41% of cases in primary care. In community cases with GE below 5 years in the USA, the prevalence of rotavirus was also low with 5% (44). Furthermore, in children hospitalized for GE in the Netherlands, norovirus and adenovirus type 41 were the second and third most detected pathogens, with 16% and 8%, respectively (41). This is in line with reports from other European countries, where norovirus is the second leading cause of hospitalizations, representing 10% to 15% of GE hospitalizations (28). Although studies from France, Italy and Austria also report adenovirus as important contributor to child GE hospitalization, prevalence rates were somewhat lower at 4% to 5% (45-47). The most common bacteria in hospitalized children in the Netherlands with GE were *Salmonella*, *Campylobacter* and *Shigella*, which were detected in 6%, 2% and 2% of children, respectively, while they were detected in less than 1% of community cases (17, 41). Likewise, *Salmonella* and *Campylobacter* are the most common bacterial agents for child hospitalization across Europe, with the most

dominant one depending on country (28, 48). In community cases below five years of age in the USA, these bacteria were also detected in less than 1% of community cases (44). Norovirus, sapovirus, *Giardia* and *Cryptosporidium* were detected just as much or more in community cases below five years in the Netherlands compared with hospitalized children. The explanation is that these pathogens are generally less likely to cause disease needing medical attention in children younger than five years compared with rotavirus, *Salmonella*, *Campylobacter* and *Shigella* (19, 49, 50).

It is important to note that pathogen infection does not necessarily lead to illness. A study among children up to 3 years of age attending DCCs in the Netherlands showed that asymptomatic infections were very common (26). The majority (78%) of more than 5,000 stool samples tested positive for at least one enteropathogen, while 95% of samples were obtained from children that had no GE. Five most commonly detected microorganisms were *D. fragilis* (22%), Enteropathogenic *E. coli* (20%), *C. difficile* (17%), norovirus (10%), and Enteroaggregative *E. coli* (5%). However, only rotavirus and norovirus were significantly associated with GE, indicating that indeed not all enteropathogen detections are clinically relevant. Interestingly, using time-series analyses to attribute these enteropathogens to the GE incidence in the DCCs, five enteropathogens were significantly associated with GE and accounted for 39% of the GE morbidity; 11% of cases were attributed to rotavirus, 10% to norovirus, 8% to *Giardia*, 7% to astrovirus and 3% to *Cryptosporidium* (51). A Danish study compared the etiology of GE in children younger than five years for which a stool sample was submitted for diagnosis of infectious diarrhea, with enteropathogens detected in an asymptomatic control group younger than five years drawn from the general population (50). They found that rotavirus, *Salmonella*, norovirus, adenovirus, *Campylobacter*, sapovirus, STEC, EPEC, *Yersinia*, and *Cryptosporidium* were associated with illness, while for eight other enteropathogens no association was found. These included attaching-and-effacing *E. coli* (A/EEC), *Giardia* and *Cryptosporodium*. The fact that an enteropathogen can be present in stool in absence of illness makes the interpretation of laboratory results challenging, particularly when multiple enteropathogens are detected in the same stool sample, or when laboratory testing is performed sequentially for groups of pathogens and discontinued when positivity is found.

Table 1. Percent prevalence of pathogen detected in stool samples of children 0 to 4 years of age with gastroenteritis in the community (n=464) (17), consulting general practitioner (n=168) (42) or admitted to hospital (n=90) (41) in the Netherlands.

	Population	General practitioner	Hospital
Year	1998-1999	1996-1999	2008-2009
Virus			
Norovirus	18	10	16
Rotavirus	10	16	54
Adenovirus 40/41	5	9	8[a]
Astrovirus	2	1	4
Sapovirus	8	5	2
Bacteria			
Salmonella	<1	7	6
Campylobacter	1	4	2
Yersinia	<1	0	0
Shigella	0	1	2
Protozoa			
Giardia lamblia	6	5	0
Cryptosporidium	3	4	3
D. fragilis	10	10	1
E. histolytica	<1	0	0

[a]Only tested for adenovirus type 41

Transmission of enteropathogens

Agents causing GE have many different transmission routes. They may spread from person-to-person (fecal-oral route), be acquired from ingested food or water, or from an environmental source, but may also result from exposure to animals (31). Once ingested, microorganisms must overcome host defensive mechanisms, including gastric acidity (which is microbicidal for most pathogens), local and systemic immune mechanisms, and gastrointestinal motility that impairs the microorganisms from adhering to the mucosa (52).

The most common transmission routes and preferred reservoirs are pathogen-specific. Non-typhoid *Salmonella* and *Campylobacter* mostly derive from animal/food and environmental sources, and infrequently spread between human (25, 31). In the Netherlands, *Salmonella* is mainly acquired from pig and poultry meat (and eggs), and *Campylobacter* mostly from poultry and cattle meat (53). About 80% of *Salmonella* infections and one-third of *Campylobacter* infections are foodborne (53). Caliciviruses (e.g. norovirus and sapovirus), rotavirus, astrovirus, adenovirus, and *Shigella* on the other hand, are indigenous to humans and spread directly between

persons or via food or water contaminated by humans. Additionally, caliciviruses may spread via vomitus (54). *C. difficile* is a major cause of nosocomial GE and spread and is more likely to occur after antibiotic use due to alteration of the bowel microflora (31, 55). *Cryptosporidium* and *Giardia* are well-known causes of waterborne-outbreaks, but may also spread from person-to-person (31). Although *Cryptosporidium hominis* is only spread between humans, *Cryptosporidium parvum* also spreads from livestock, especially cattle, to humans (56). For *Giardia,* which also has animal reservoirs such as dogs and cats, the zoonotic potential has never been proven conclusively (56). The large differences in modes of transmission and preferred reservoir(s) between enteropathogens emphasize the importance of control measures to be tailored towards the pathogen in question.

The likelihood of pathogen spread depends on the concentration of microorganisms in excreta, the survival and replication capacity of the microorganisms in food or persistence in the environment, the infectious dose, and hygiene (31). Closed environments are especially prone to spread of microorganisms, such as DCCs, schools, and cruise ships (18). A study on GE outbreaks with suspected viral etiologies from 1994 through 2005 in the Netherlands described a total of 923 outbreaks that were reported to local health authorities in the Netherlands, of which 55 (6%) occurred in a DCC and 19 (2%) in a school (57). These numbers are likely an underestimation, because in settings other than residential institutions and hospitals, reporting of GE outbreaks is not obligatory, and outbreaks with bacterial and protozoan etiology were not included. Household transmission between siblings and from children to their parents and other family members is common, because preschool children are often exposed to other children (e.g. in day-care), have imperfect personal hygiene, an immature immune system, and require more hands-on care by their parents (58). A retrospective cohort study among parents and their children aged 12 – 60 months in Canada found that transmission of GE in children to their parents occurred once every three GE episodes (58).

Knowledge gaps

The last estimate of community GE in the Netherlands dates back to 2009 (20). In order to monitor possible changes in the burden of GE, an update was needed for the 2010s. Moreover, previous studies did not explicitly consider the role of children in determining household differences in GE burden, as well as the associated risk factors, while the incidence is known to be highest in (families with) children and likely represent a substantial part of the GE burden in the community (58). These

data would help identifying potential targets for control initiatives to reduce the burden of GE. Furthermore, previous estimates of GE-associated societal costs in the Netherlands also originate from 2009 but likely underestimate the current costs because healthcare costs have increased substantially in the meantime (59). Likewise, no stratification was made according to the presence of children in the household, while previous research has shown that illness in young children leads to substantial productivity loss due to work absenteeism of their parents. A study among families with children below five years in the Netherlands found that one and a half day of paid worktime were missed, on average, per each GE episode of the child (18). Cost-of-illness studies provide essential input for economic evaluations of possible intervention measures to support decision-making in public health. They also allow for comparison of the relative burden of different (infectious) diseases with one another, as well as over different groups of the population, geographical regions, and periods.

There are few comprehensive studies on the viral, bacterial and protozoan etiology of GE in the community. Most studies focus on patients presenting to the GP or admitted to hospital, which is a nonrandom selection of GE cases including only cases requiring medical attention. Because disease-severity is enteropathogen-dependent and herewith healthcare seeking behavior, these studies unlikely reflect the etiology of GE in the general population. Two prospective population-based studies in the United Kingdom during 1993–1996 and 2008–2009 found a substantial reduction in *Salmonella* incidence as a result of the Europe-wide successful intervention of the *Salmonella* control programme in poultry, and an increase in the detection of norovirus and sapovirus (60, 61). Because the last study on the etiology of GE in the general Dutch population was conducted already two decades ago, an update is required to determine whether changes in the etiology of GE also occurred in the Netherlands (17). Moreover, these data are needed to update the relative contribution of different pathogens to GE, which is essential in the interpretation of positive laboratory results.

It is still unclear whether children with co-infections are at increased risk for (more severe) GE, as evidence in literature is conflicting (45, 62-65). Four out of ten children hospitalized for GE in the Netherlands had co-infections of viruses, bacteria and parasites (41). Because most studies on the clinical relevance of co-infections focus on children that are hospitalized, they can only assess disease severity (45, 62-65). However, they cannot determine whether children with co-infections more often have GE compared to children infected with a single enteropathogen.

For this purpose, studies comparing the etiology in children with and without GE are required, but are scarce in literature. Furthermore, interactions between enteropathogens could influence whether enteropathogens occur completely at random or depend on the presence of other microorganisms as well, but these are not well understood (66).

Currently, transmission dynamics and targets for control in DCCs, where children are at increased risk for transmission compared to their home-cared counterparts, are not well understood (18). A previous study in DCCs in the Netherlands identified several socio-economic, facility- and policy-related DCC characteristics associated with the prevalence of enteropathogens as a whole (67). Risk factors included, among others, the number of attending children, having animals in the DCC, allowing mixing of staff members between child groups, and protective factors were disinfecting fomites with chlorine, daily cleaning of bed linen/toys, and cohorting and exclusion policies for ill children. However, risk factors for sporadic occurrence of enteropathogens may differ from the ones for temporally clustered occurrence (e.g. seasonality, outbreaks) of these enteropathogens, and hereby strategies for invention. Furthermore, pathogen-specific knowledge gaps also exist. Notably, despite several studies have reported epidemiological differences between *G. lamblia* assemblages, which contribute to 8% of GE morbidity in DCC-attending children, knowledge of assemblage-specific transmission routes and reservoirs (which may well be either zoonotic or anthroponotic in nature) remain limited (51). For example, two studies conducted in England and Malaysia found that keeping pets (e.g. dogs) was associated with assemblage A infection, while assemblage B was associated with the presence of children in the household and other members in the family with diarrhea (68, 69). In line with these findings, assemblage A is more often detected in companion animals than assemblage B, and is therefore thought to have greater zoonotic potential (56). However, evidence to support this hypothesis is inconclusive.

Another pathogen-specific knowledge gap concerns rotavirus. Indeed, despite the absence of rotavirus vaccination as part of the national immunization programme in the Netherlands, an unexpected drop in the number of rotavirus laboratory detections in a sentinel network of laboratories was observed in 2014. A hyper-endemic year was expected in the following year(s) due to an accumulation of susceptible children. However, the rotavirus season was as usual in 2015, followed by another unexpected drop in the number of laboratory detection in 2016, suggesting a possible biannual pattern. It was hypothesized that these drops were possibly

due to a milder course of the disease, and that a shift occurred towards the older children after the 2014 and 2016 low-endemic years due to an accumulation of susceptible children. Understanding the underlying mechanisms of this unusual rotavirus pattern is essential to be able to estimate the effect of rotavirus vaccine implementation in 2019 on the burden of rotavirus GE.

Thesis aims

This thesis aims to fill the aforementioned knowledge gaps, thereby providing answers to questions that have arisen from previous research and have remained unanswered, using mainly existing data sets from previously conducted studies. For example, previous studies assessed the incidence, severity, societal costs and associated risk factors of GE in children attending DCCs in the Netherlands, but whether temporal clustering of these enteropathogens in DCCs occurs and exhibits specific risk factors has not been studied (70). The aim of this thesis is to increase our understanding of the epidemiology of GE in preschool children and understand their role in the household burden of GE. First, we aim to estimate the burden and correlates of GE as syndrome and of specific enteropathogen infections in the Netherlands' general population, with special emphasis on families with preschool children. Second, we aim to elucidate the etiology of GE and clinical relevance of the different enteropathogens circulating in preschool children and their parents. Third, we aim to unfold epidemiological patterns in determinants, clustering, temporal trends and risk factors of some of the most important enteropathogens in children, with special attention to DCCs as 'transmission setting', and to *Giardia* as pathogen in a separate account. Lastly, we aim to quantify and understand the unexpected epidemiological pattern of rotavirus in the Netherlands since 2014.

Gastroenteritis burden in the general population and the role of preschool children

In **Chapter 2**, data from a large monthly-repeated cross-sectional study named "ESBL attribution project" (ESBLAT) is used to estimate the incidence of GE in the Netherlands in all age groups, stratifying results by families with and without young children (71). We also identify risk factors for GE in all age groups, and estimate the economic burden of GE. **Chapter 3** describes another large monthly-repeated cross sectional study focusing on preschool children and their parents called "Family & Health" (Dutch name: "Gezin & Gezondheid"). It elucidates the burden and risk factors for GE in families that have young children, as well as the societal burden of GE in these families. It also describes the role of DCC attendance in the household burden of GE.

Prevalence, risk factors, clinical relevance and household transmission of enteropathogens in preschool children

Chapter 4 uses microbiological stool sample data of child-parent pairs from the Family & Health study, which were sampled irrespective of symptoms, in order to determine the prevalence and risk factors of a panel of 19 enteropathogens. This chapter addresses the clinical relevance of these pathogens with regard to GE and associated risk factors, and assesses to what extend household transmission between the child and their parent occurs. C**hapter 5** builds on the previous chapter and describes whether co-infections occur by chance (i.e. randomly) or whether enteropathogens might possibly influence each other's occurrence. Moreover, it determines whether children with co-infections are more likely to experience GE compared with children with a single infection.

Clustering of enteropathogens in day-care centers and the potential for zoonotic transmission

Chapter 6 describes the temporal clustering of five major enteropathogens (rotavirus, norovirus, astrovirus, *Giardia lamblia*, and *Cryptosporidium*) in DCC attendees, and identifies risk factors for such clustering. For this purpose, we used data from a nationwide prospective cohort study in DCCs named "KIzSS". In **Chapter 7**, we also use these data to describe assemblage-specific risk factors for *G. lamblia* assemblages A and B infection in DCC-attending children.

Quantifying the unexpected decrease in rotavirus laboratory detections in the Netherlands

Chapter 8 uses data from two population-based studies (i.e. KIzSS and Family & Health) and three passive surveillance systems (i.e. rotavirus laboratory detections, GE consultations data from a GP network, and GE hospitalization data). These data were used to assess whether the unexpected drop in the number of rotavirus laboratory detections were the result of a less severe course of the disease or a genuine drop in rotavirus circulation. **Chapter 9** explores whether a shift in age distribution of rotavirus GE occurred in children younger than five years following the low-endemic 2014 year. For this purpose, updated data on GE consultations from the same GP network as in chapter 8 is used as well as rotavirus laboratory detections from a sentinel network of laboratories.

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CHAPTER 2

Incidence and economic burden of community-acquired gastroenteritis in the Netherlands: Does having children in the household make a difference?

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Submitted to PLOS ONE

Abstract

Background

This study aimed at estimating gastroenteritis (GE) incidence in all age groups of the Netherlands' general population, with special emphasis on the role of children in GE burden, and the associated costs.

Methods

Monthly from November 2014 to November 2016, a random sample of 2000 residents in the Netherlands was invited to complete a questionnaire on household characteristics and health complaints. We calculated GE incidence rates standardized to the Dutch population and used multivariable logistic regression models to identify potential risk factors. We calculated the costs related to resources used within the healthcare sector, the resources used by patients and their families, and productivity losses (paid worktime) due to GE.

Results

The overall standardized incidence rate was 0.81 GE episodes/person-year, with the highest rate in children ≤4 years (1.96 episodes/person-year). GE was observed more often in households with children (≤17 years), especially if children attended out-of-home childcare services, and among individuals with non-native Dutch ethnic background. Less GE was observed among employed persons aged 25–64 years, compared with those unemployed, but the opposite was observed in persons ≥65 years. The average costs per GE episode was €191, resulting in €945 million annual total costs for GE in the Netherlands (€55 per inhabitant). The majority of costs (55%) were attributable to productivity losses of the ill or their caregivers.

Conclusions

GE still poses a significant burden, particularly in preschool children and adults living in households with children. Similar to other industrialized countries, the major factor driving the costs due to GE was the loss of productivity. This study also provides up-to-date baseline GE incidence rates and associated societal costs to better contextualize the burden of the disease in support of policy making.

Introduction

Gastroenteritis (GE) ranks first in deaths caused by communicable diseases globally, although the numbers have reduced over the past decades [1]. In industrialized countries, however, the course of the disease is often mild and short-term [1-3]. Despite being rarely fatal, the morbidity and economic costs of GE are substantial due to its high incidence even in an industrialized country like the Netherlands [3-5]. Cost-of-illness (COI) studies are instrumental in public health policy and serve as important input for economic evaluations of possible intervention measures to support decision-making. They namely measure the costs related to resources used within the healthcare sector, the resources used by patients and their families, and productivity losses and other non-healthcare related resources used that are indirectly related to illness [6]. They highlight the magnitude of the impact of an illness on the society and allow for comparison of the relative burden of different (infectious) diseases.

In the Netherlands (~17 million population), the average costs of each GE episode was estimated at €77 in 2003, with total national costs of €345 million [4]. In 2009, the average costs per GE episode had increased to €133–€151, corresponding to national costs of €611–695 million [3]. This increase was mainly attributable to a rise in healthcare costs over the years. These two studies were based on GE incidence estimates from a prospective population-based cohort study conducted in 1999, reporting 283 GE episodes per 1,000 person-years [7]. The incidence was estimated to be more than 3 times higher in a retrospective population-based cohort study in 2009, with 964 GE episodes per 1,000 person-years [2]. This large difference was thought to be mainly due to the retrospective nature of the latter study, which was more sensitive to 'telescoping' bias, i.e. remembering a GE episode to be more recent than it actually was, and sample selection bias of those who recently experienced GE. It is likely that the average costs per GE episode are currently higher than was found in 2009 because healthcare costs have further increased [3, 6]. Since 2009, studies have focused on GE in young children and/or their parents in the Netherlands, and none estimated the GE incidence in all age groups [8, 9]. Moreover, previously published COI studies on GE in the Netherlands did not differentiate between households with and without children, whilst the incidence of GE is known to be highest in households with young children [9, 10], with parents and siblings of GE-affected children having an up to 4- and 8-fold increased risk for secondary GE, respectively [9, 11].

The overall aim of this study was to update and characterize estimates for community-acquired GE in all age groups of the general population in the Netherlands, with special emphasis on the role of children in GE burden in the household, including associated costs. This was achieved by (i) estimating GE incidences, (ii) identifying risk factors for GE, (iii) identifying differences in GE burden between households with and without children, and (iv) estimating the average economic costs of GE episodes.

Materials and methods

Study design

We used questionnaire data that was collected during a monthly-repeated cross-sectional survey on antimicrobial resistance that was performed among the general population in the Netherlands from November 2014 to November 2016 [12]. A random sample of 2000 Dutch residents, including all age groups, was monthly drawn from municipal population registries covering the whole national population with a maximum of one person per household. They were invited by regular mail to complete a web-based questionnaire. Invitees could fill in the questionnaire with the help of someone else, such as a family member or a healthcare worker. For children aged 0–12 years, a parent or caregiver was asked to fill out the questionnaire. Children aged 13–17 years could fill in the questionnaire themselves, but help from a parent was recommended.

Questionnaire

The web-based questionnaire included questions about general demographics (e.g. age, gender, country of birth), education, employment, household characteristics (e.g. number of children, age of children in the household, presence of pet), out-of-home care of children (i.e. day-care attendance, kindergarten, guest parent), outdoor activities, health symptoms in the past four weeks (e.g. diarrhea, vomiting), chronic conditions (e.g. enteropathies, food allergy, immune disorder) the use of medication (e.g. antibiotics, antacid drugs, paracetamol), medical care (e.g. contact with the general practitioner (GP), hospitalization) and laboratory testing.

Gastroenteritis case definition

A GE case was defined as a person with ≥3 diarrheal discharges or ≥1 episode of vomiting in 24 hours during the four weeks prior to completing the questionnaire, according to a commonly used GE case definition [13]. We excluded cases of probable non-infectious origin, i.e. those with underlying enteropathies. The same case definition was also used in previous studies estimating the incidence of GE in the general population in 2009 in the Netherlands, with the exception that we were not able to exclude cases with vomiting due to regurgitation, motion sickness/vertigo, nauseous event, traumatic event, or drug/alcohol abuse, as this information was not collected [2, 12].

Statistical analysis

The representativeness of the sample of study participants was assessed in relation to the demographics of the Dutch general population, including age (five-year age groups; 0–4, 5–9, 10–14 years etc.), gender, and location of residence (urban: ≥2500 households/km^2, intermediate: 500 – 2500 households/km^2, rural: <500 households/km^2). The cut-offs for location of residence were those adopted by the Dutch Central Bureau for Statistics (CBS) [14]. All data analyses were weighted to adjust for differences in the distributions by age, gender and location of residence between our study population and the general population the sample was drawn from.

The incidence of GE was expressed as the number of episodes per person-year (365/28 x 4-weekly GE incidence proportion), because the recall period was 28 days. GE incidence was reported by age groups (0–4, 5–17, 18–29, 30–44, 45–65, and 65+ years of age), gender, location of residence, and level of education. Age groups cut-offs were chosen according to previous cost-of-illness studies on GE in the Netherlands to ensure comparability [2, 3]. Age groups 18–29 ('young adults'), 30–44 ('adults') and 45–65 ('middle-aged') years were stratified by the presence of children (defined as those aged 0–17 years) in the household to study differences between households with and without children. For all adult groups (defined as those aged ≥18 years) living in a household with children, no data was available on whether they were the parents of those children, or for example an older sibling or another relative. The level of education was categorized as low (primary, lower vocational or lower secondary education), intermediate (intermediate vocational, intermediate secondary or higher secondary education), and high (higher vocational and university education). For those younger than 18 years who did not fill in the questionnaire themselves, the education level of the person filling it in was used.

An overall multivariable weighted logistic regression model was built to assess the effect of age (0–4, 5–17, 18–29, 30–44, 45–65, and 65+ years of age), gender, location of residence (rural, intermediate and urban), and educational level (low, intermediate, high) on GE. In addition, a multivariable weighted logistic regression model was built for each age group to assess the effect of the number of children in the household (one, two, or ≥three children, with 'no children' as reference group), the age of children in the household (0, 1–2, 3–12, and 13–17 years) and their attendance of out-of-home care (i.e. day-care, kindergarten, guest parent, with "no out-of-home-care" as reference group) on GE. Location of residence, educational level, employment, gender, country of birth (born in- or outside the Netherlands), and comorbidities (food allergy, immune disorder, asthma, and cancer) were considered as potential confounders. They were kept in the model when a change of more than 10% in the coefficients of the other covariates was observed when removed from the model, regardless of significance. We also tested variables for biologically plausible interactions as reported previously [9]. Selection between collinear variables was based on improved model fit using the Akaike information criteria (AIC). A backward stepwise variable procedure was applied, where variables with a p-value <0.05 were kept in the model. Associations were expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI). Analyses were performed using STATA version 15.1 (College Station, TX, USA).

Cost of illness

Costs of GE were divided into direct healthcare costs, patient and/or family costs and productivity losses, as described below. The average costs per GE episode, the average costs per Dutch inhabitant (total costs of GE/17.2 million inhabitants), as well as the total costs of GE, were calculated for age groups 0–4, 5–17, 18–29, 30–44, 45–65, and 65+ years of age. For age groups 8–29, 30–44 and 45–65 years, costs were further stratified by households with and without children. Unit costs were expressed in 2017 Euros (€) (S1 Table). Because retrospective surveys on disease burden are prone to overestimation of the incidence, we adjusted our GE estimates when calculating costs, based on findings from a community study on GE in the United Kingdom.[2, 15-20]. They found a GE estimate of 55/100 person-years in a retrospective survey, while it was 19.4/100 person-years (2.8 times lower) in a prospective study in the same study population [21]. Hence, to remain conservative, we divided all our GE estimates by a factor of 2.8 when calculating the average costs per inhabitant (regardless of GE) and the total costs. However, we also calculated

the upper boundary of costs without applying the correction factor. The rationale is that over-selection of persons with GE might be less pronounced in our study than the retrospective study in the United Kingdom, because participants in our study were invited to participate in a study on antibiotic resistance and not on GE. Hence, persons with GE might have been equally motivated to participate in our study compared with persons without GE. Moreover, prospective studies are also subject to biases, including selection bias, which can potentially lead to under- or overestimation of the incidence, which affects the correction factor as well [22]. We report GE estimates without correction in order to ensure comparability with previous studies on GE community incidence, which were mainly retrospective in nature [2, 15-20].

Direct healthcare costs

Direct healthcare costs included costs for GP consultation, hospitalization, ambulance transport to the hospital, medicines prescribed by a doctor, and laboratory testing. Because we had data on whether respondents contacted the GP, but not whether they actually visited the GP, we used data on overall all-cause GE consultation rates in GPs in 2016 to scale the GP consultation rate in our study. These data were collected by routine electronic health record extractions from Dutch GPs participating in the Nivel Primary Care Database (~7% national coverage) [23]. Since the questionnaire collected no data on ambulance utilization and length of hospitalization, we used data from a previous study in patients hospitalized for GE in the Netherlands [3, 24]. They reported that 1% of hospitalized children younger than 18 years of age and 50% of hospitalized adults were transported to hospital by ambulance. The mean length of hospitalization was 2.9 days in children, 9.9 days in adults 18–64 years, and 12.3 days in persons 65+ years. For the first GP visit, we used a weighted cost consisting of a standard consultation (i.e. 90% of patients visiting the GP and in 10% of the cases a house visit was required), and an additional telephone consultation (97% of all GP visits) based on previous research [25]. For subsequent visits, we assumed only the costs for the standard consultation. Medicine costs were derived from retail prices and prescription drug dispensing fees in the official price list published by Care Institute Netherlands (in Dutch: 'Zorginstituut Nederland') [26]. The duration of medicine use was based on the recommendations in the package leaflet.

Patient and/or family costs

Patient and/or family costs were costs paid by the patients themselves and/or his family, and included travel costs to the GP or hospital, as well as over-the-counter (OCT) medicines. Based on previous research, we assumed that 77% of persons younger than 15 years and 97% of those older than 15 years used a car or public transport (50/50) to go to the GP [3]. We used the Dutch average distance of 1.1 kilometers to the GP and 7.0 kilometers to the hospital, as well as a parking fee when traveling by car [6]. Similar to medicines prescribed by a doctor, we derived the costs of OCT medicines from the official retail price list of Care Institute Netherlands, but without pharmacist dispensing fees [26].

Productivity losses

Productivity losses were costs of time spent away from paid employment by the patients themselves and/or their caregivers as a result of a GE episode. Because our study collected no data on absenteeism from work, we based the number of paid work hours missed per GE episode on previous Dutch studies [3, 24]. In children younger than 5 years, two hours of paid work were missed per GE episode for caregiving by their parents or a relative [3]. In the age group 18–64 years, 4.5 work hours were missed for each GE episode by themselves or a caregiver [3]. In persons 65 years and older, 0.4 hours of paid work were missed by a caregiver [24]. For children aged 5–17 years, in absence of available literature, we assumed that the amount of paid employment missed was 50% of that missed in children younger than 5 years, i.e. 1 hour per GE episode, in line with assumptions from previous research [24]. The hourly wage of paid employment was obtained per five-year age group (i.e. 15–19 years, 20–24 years etc.) [6, 27, 28].

Costs were expressed in 2017 euros, and where necessary, updated to 2017 using Dutch consumer Price index as reported by the Dutch Central Bureau for Statistics [29].

Results

In total, 9,512 of the 48,024 invited individuals (response: 19.8%) filled in the questionnaire. Questionnaires of 256 participants were discarded because they were incompletely filled in regarding the variables used here, leaving 9,256 participants. Compared to the general population, our sample overrepresented females (our study

population: 52.6% *vs.* general population: 50.2%) and individuals from rural areas (31.7% *vs.* 16.3%), and underrepresented individuals from urban areas (6.2% *vs.* 22.7%) (S2 Table). Moreover, the mean age in our study population was higher than in the general population (42.7 years *vs.* 41.5 years). These differences were adjusted for by performing weighted analyses.

Gastroenteritis incidence

A total of 544/9,256 persons (5.9%, standardized rate: 6.2%, 95%CI 5.6–6.9) experienced GE in the four weeks before completing the questionnaires. We excluded 97 persons (15.1%) with GE because they suffered from underlying enteropathies. Of the 544 persons with GE, 69 (12.7%) had diarrhea and vomiting, 257 (47.2%) had diarrhea without vomiting, and 218 (40.1%) had vomiting without diarrhea. The most frequently reported additional symptoms were abdominal cramps (43.6%), nausea (39.7%), fever (18.9%), mucus in stool (4.8%), and blood in stool (2.4%).

The overall standardized incidence was 0.81 GE episodes per person-year (95%CI: 0.72-0.90) (Table 1). The incidence was highest in children ≤4 years (1.96 episodes/person-year, 95%CI: 1.51-2.42), and lowest incidence in persons aged 45–65 years (0.60 episodes/person-year, 95%CI: 0.48-0.71) and ≥65 years (0.46 episodes/person-year, 95%CI: 0.32-0.60).

Risk factors for gastroenteritis

In children ≤4 years of age, living in a household with other children of <1 year was a risk factor for GE (OR 3.2, 95%CI: 1.2-8.7) compared with having no other children in the household, whereas living in an urban area was associated with a lower risk (OR 0.2, 95%CI 0.1-0.9) (Table 2). In children aged 0–17 years, having an immune disorder or asthma were risk factors for GE (OR 8.4, 95%CI: 1.5-47 and OR 8.1, 95%CI 2.3-29, respectively).

In (young) adults in the age group 18–24 years, risk factors were living in a household with children aged 3–12 years (OR 25.6, 95%CI: 3.9-168), compared with living in a household without children (Table 3). In the age group 25–44 years, those living in a household with a child aged 3–12 years and at least one child attending out-of-home care, had more GE than those living in a household without children (OR 2.8, 95%CI 1.1–7.3). Non-native Dutch males also had more GE compared to native Dutch males (OR 4.0, 95%CI: 1.1-15).

Table 1. Incidence of gastroenteritis per person-year by sociodemographic variables in the general population in the Netherlands, November 2014 to November 2016

	N	GE	Crude %	St.[a] %	95%CI	GE/yr	95% CI	aOR[b]	95% CI
Age in years									
0-4	522	86	16.5	15.1	11.9-19.0	1.96	1.51-2.42	2.6	1.8-3.8
5-17	1,439	98	6.8	6.3	4.9-7.7	0.82	0.64-1.00	0.9	0.6-1.3
18-24	555	56	10.1	11.3	7.5-15.2	1.47	0.98-1.98	1.8	1.1-3.0
With children[c]	143	14	9.8	13.2	4.7-21.7	1.72	0.61-2.83	n.i	
Without children[c]	411	42	10.2	10.8	6.5-15.1	1.41	0.85-1.97	n.i	
25-44	1,784	109	6.1	6.5	4.9-8.1	0.85	0.64-1.06	Ref	
With children[c]	1,018	65	6.4	7.0	4.7-9.2	0.91	0.61-1.20	n.i	
Without children[c]	758	44	5.8	6.0	3.6-8.5	0.78	0.47-1.11	n.i	
45-64	2,915	132	4.5	4.6	3.7-5.5	0.60	0.48-0.71	0.7	0.5-0.9
With children[c]	799	28	3.5	3.6	2.2-5.0	0.46	0.28-0.65	n.i	-
Without children[c]	2,097	103	4.9	5.0	3.9-6.1	0.65	0.50-0.80	n.i	-
65+	1,874	61	3.3	3.5	2.6-4.8	0.46	0.32-0.60	0.5	0.3-0.7
Gender									
Male	4,389	241	5.5	5.9	5.0-7.0	0.77	0.64-0.90	Ref	-
Female	4,867	303	6.2	6.5	5.7-7.5	0.85	0.73-0.96	1.1	0.9-1.4
Location of residence									
Rural	2,937	169	5.8	5.4	4.7-6.3	0.71	0.60-0.81	Ref	-
Intermediate	5,747	336	5.9	6.1	5.5-6.8	0.79	0.71-0.88	1.1	0.9-1.4
Urban	572	39	6.8	7.1	5.2-9.8	0.93	0.63-1.23	1.2	0.8-1.8
Education level									
Low	2,933	164	5.6	5.9	4.9-7.1	0.77	0.63-0.91	Ref	-
Intermediate	2,929	179	6.1	6.4	5.3-7.7	0.84	0.68-0.99	0.8	0.6-1.1
High	3,160	183	5.8	6.0	5.0-7.2	0.78	0.64-0.94	0.8	0.6-1.1
Overall	9,256	544	5.9	6.2	5.6-6.9	0.81	0.72-0.90	-	-

GE: gastroenteritis, 95%CI: 95% confidence intervals, aOR: adjusted odds ratio, n.i.: not included in the multivariable model[a]. Standardized by gender, age (five-year age groups) and location of residence (urban, intermediate, rural); [b]Adjusted for age, gender, location of residence, and educational level; [c]Children aged 0–17 years living in the same household;

In the age groups 25–44 years and 45–64 years, employed persons with a high educational level had fewer episodes of GE compared with those unemployed (OR 0.4, 95%CI: 0.1-0.9 and OR 0.4, 95%CI: 0.2-0.9, respectively). In the age group 45–64 years, the same was observed for employed persons with an intermediate educational level compared with unemployed persons (OR 0.5, 95%CI: 0.3-1.0).

In persons 65 years or older, females with and without native Dutch ethnic background had more GE compared with native Dutch males (OR 6.4, 95%CI: 2.5-17 and OR 2.1, 95%CI: 1.1-4.2). Those with a food allergy (OR 3.1, 95%CI: 1.2-8.4) or cancer (OR 3.1, 95%CI: 1.5-6.4) also had more GE. Employed persons with a low educational level had more GE compared with unemployed or retired persons (OR 4.6, 95%CI: 1.1.-20).

Table 2. Multivariable weighted logistic regression models of factors associated with gastroenteritis in children, stratified by age groups

	0 – 4 years (n=493)			**5 – 17 years** (n=1,296)		
	N (%GE)[a]	**OR[a]**	**95%CI**	**N (%GE)**	**OR[a]**	**95% CI**
Other children in household						
None	146 (10)	Ref		171 (6)	Ref	
0y	63 (23)	**3.2**	**1.2-8.7**	25 (9)	0.8	0.2-3.4
1-2y	71 (16)	2.0	0.8-4.7	37 (13)	1.0	0.3-3.5
3-12y	214 (14)	1.3	0.7-2.4	655 (7)	0.8	0.4-1.6
13-18y	7 (4)	0.8	0.1-7.3	408 (4)	0.6	0.2-1.6
Out-of-house care[b]		-			1.7	0.8-3.7
Location of residence						
Rural	177 (18)	Ref		490 (7)	Ref	
Intermediate	303 (17)	1.0	0.6-1.6	882 (7)	0.9	0.6-1.5
Urban	42 (10)	**0.2**	**0.1-0.9**	67 (3)	0.4	0.1-1.9
Comorbidities						
Cancer	1 (0)			3 (0)		
Food allergy	14 (2)			35 (7)		
Immune disorder[c]	1 (0)			6 (33)	**8.4**	**1.5-47**
Asthma	2 (100)	n.c.		12 (27)	**8.1**	**2.3-29**

GE: gastroenteritis, OR: odds ratio, 95% CI: 95% confidence interval, n.c.: non calculable
[a]Standardized by gender, age (five-year age groups) and location of residence (urban, intermediate, rural); [b]Day-care, kindergarten or guest parent attendance of other children in the household; [c]E.g. Guillain Barre Syndrome, Grave's disease, Addison's disease, sarcoidosis

Table 3. Multivariable weighted logistic regression models of factors associated with gastroenteritis in adults, stratified by age groups

	18 – 24 years (n=555)			25 – 44 years (n=1,784)			45 – 64 years (n=2,892)			65+ years (n=1,874)		
	N (%GE)	OR[a]	95%CI	N (%GE)	OR[a]	95%CI	N (%GE)	OR[a]	95%CI	N (%GE)	OR[a]	95%CI
Children in household												
No children	411 (11)	Ref		758 (6)			2,262 (5)	Ref		1,863 (3)	-	
0y	1 (0)	n.c.		61 (13)			5 (0)	n.c.		0		
1-2y	2 (0)	n.c.		86 (7)			5 (0)	n.c.		0		
3-12y	7 (68)	**25.6**	**3.9-168**	470 (6)			123 (6)	1.2	0.5-2.8	1 (0)		
13-17y	109 (12)	1.4	0.5-3.9	80 (2)			507 (3)	0.6	0.3-1.2	4 (0)		
Children x daycare[b]												
No children	411 (11)	-		758 (6)	Ref		2,262 (5)	-		1,863 (3)	-	
No daycare, 0y	0			28 (14)	3.7	0.4-32	4 (0)			0		
Daycare, 0y	1 (0)			33 (11)	3.0	0.5-19	1 (0)			0		
No daycare, 1-2y	1 (0)			22 (4)	0.9	0.1-6.3	3 (0)			0		
Daycare, 1-2y	1 (0)			64 (8)	1.8	0.6-5.3	2 (0)			0		
No daycare, 3-12y	6 (78)			310 (4)	0.7	0.2-2.1	100 (6)			1 (0)		
Daycare, 3-12y	1 (0)			160 (10)	**2.8**	**1.1-7.3**	23 (7)			0		
13-17y	109 (12)			80 (2)	0.2	0.0-1.3	507 (3)			4 (0)		
Gender x migration[d]												
Dutch male	214 (11)	-		700 (5)	Ref		1,308 (4)	-		977 (2)	Ref	
Non-Dutch male	9 (11)			50 (16)	**4.0**	**1.1-15**	70 (1)			70 (1)	0.4	0.1-3.5
Dutch female	324 (12)			981 (7)	1.1	0.6-2.3	1,587 (5)			768 (4)	**2.1**	**1.1-4.2**
Non-Dutch female	8 (16)			53 (3)	0.3	0.0-2.2	117 (11)			59 (13)	**6.4**	**2.5-17**
Employment x education												
No employment	225 (6)	Ref		229 (8)	Ref		803 (6)	Ref		1,580 (3)	Ref	
Yes, low	44 (5)	0.5	0.1-2.8	194 (6)	0.8	0.2-2.5	615 (5)	0.9	0.5-1.7	24 (13)	**4.6**	**1.1-20**

Table 3. Continued.

	18 – 24 years (n=555)			25 – 44 years (n=1,784)			45 – 64 years (n=2,892)			65+ years (n=1,874)		
	N (%GE)	**OR[a]**	**95%CI**	**N (%GE)**	**OR[a]**	**95%CI**	**N (%GE)**	**OR[a]**	**95%CI**	**N (%GE)**	**OR[a]**	**95%CI**
Yes, intermediate	198 (15)	1.7	0.6-4.8	536 (6)	0.5	0.2-1.2	755 (3)	**0.5**	**0.3-1.0**	14 (0)	n.c.	
Yes, high	73 (12)	3.1	0.9-10	784 (6)	**0.4**	**0.1-0.9**	795 (3)	**0.4**	**0.2-0.9**	22 (0)	0.9	0.1-6.8
Comorbidities												
Cancer	0	-		18 (10)	-		114 (1)	-		137 (9)	**3.0**	**1.4-6.5**
Food allergy	28 (24)	-		68 (5)	-		64 (5)	-		29 (14)	**3.5**	**1.3-9.3**
Immune disorder[c]	10 (11)	-		26 (9)	-		42 (5)	-		22 (0)	-	
Asthma	5 (26)	-		12 (0)	-		18 (9)	-		0	-	

GE: gastroenteritis, OR: odds ratio, 95% CI: 95% confidence interval, n.c.: non calculable

[a]Standardized by gender, age (five-year age groups) and location of residence (urban, intermediate, rural);

[b]Day-care, kindergarten or guest parent attendance of at least one child in the household; [c]E.g. Guillain Barre Syndrome, Grave's disease, Addison's disease, sarcoidosis; [d]Born in- or outside the Netherlands

Costs per gastroenteritis episode

An estimated 123/544 GE cases (6.3%, standardized: 6.4%) visited the GP at least once, with an average of 0.13 visits per GE episode (standardized: 0.12 visits), and 9/544 cases required hospitalization (1.7%, standardized: 1.6%). Seven cases (1.7%, standardized: 1.3%) reported that stool sample tests were performed (Table 4). Medicines were prescribed by a doctor in 43 cases (8.7%, standardized: 7.8%) and bought OCT by 193 cases (35.8%, standardized: 35.3%).

The average costs per GE episode was €126 in children 0–4 years, €61 in the age group 5–17 years, €82 in the age group 18–24 years, €182 in the age group 25–44 years, €261 in the age group 45–64 years, and €475 in persons ≥65 years (Table 5). The majority of overall costs were due to absence from paid work (55%) and hospitalization (36%). Productivity losses (i.e. the costs due to absence from paid work) were highest in the age group 40–65 years and hospitalization costs were highest in those ≥65 years. Patient costs accounted for only 2% of the overall costs.

With overall average costs per GE episode of €191 and an adjusted incidence of 0.29 episodes/person-year, the estimated costs of GE for the Netherlands in 2017 amounted to €945 million. Without correction for potential overestimation of our incidence, the upper boundary of the costs would be €2.6 billion. When averaging the total costs over the total number of inhabitants, the costs were €55 per inhabitant per year (€945 million/17.2 million inhabitants), and €154 as upper boundary (€2.6 billion/17.2 million inhabitants). The average costs per inhabitant aged 18–64 years were similar for those living with and without children in the household, namely €53 and €55, respectively. However, differences were observed when further stratifying by age. In the age groups 18–24 years and 25–44 years, costs were higher in those living with children in the household than those living without children in the household (18–24 years: €50 and €41, respectively; 25–44 years: €60 and €51, respectively). However, in the age group 45–64 years, costs were lower in those living with children in the household than those living without children in the household (€43 and €61, respectively).

Table 4. Average use of resources per gastroenteritis case, by age group

Average use per GE case	**0-4** (n=522)	**5-17** (n=1,439)	**18-24** (n=555)	**25-44** (n=1,784)	**45-64** (n=3,082)	**65+** (n=1,874)	**All ages** (n=9,256)
Direct healthcare costs							
GP visit	0.13	0.06	0.07	0.12	0.11	0.26	0.12
Hospitalization days	0.07	0.03	0.00	0.05	0.12	0.83	0.14
Medicine prescription	0.17	0.02	0.08	0.09	0.09	0.15	0.09
Stool sample laboratory test	0.03	0.01	0.01	0.00	0.03	0.00	0.01
Patient costs							
Car//bus transport	0.10	0.06	0.07	0.12	0.11	0.26	0.12
Ambulance transport	<0.01	<0.01	0.00	<0.01	<0.01	0.05	0.01
Medicine OCT	0.29	0.40	0.44	0.44	0.49	0.36	0.42
Productivity losses							
Hours absence paid work[a]	2	1	4.5	4.5	4.5	0.2	3.0

GE: gastroenteritis, GP: general practitioner, OCT: over-the-counter
[a]Of themselves or a caregiver

Discussion

This study provides updated estimates of the incidence of GE in different strata of the Dutch community and the first estimates of such incidence in households with and without children. The overall incidence was estimated at 0.81 GE episodes per person-year, with the highest incidence in children aged 0–4 years and the lowest one in those aged ≥65 years. The average costs per GE episode were estimated at €191, corresponding to a total economic burden of €945 million in 2017. Without correction for potential overestimation of the GE incidence, the costs would be €2.6 billion.

The observed GE incidence was slightly lower than the previous estimate of 0.96 GE episodes per person-year in the Netherlands in 2009 [2]. In general, retrospective studies in other European countries have found higher GE incidence rates, namely 0.9 GE episodes/person-year in Poland, 1.1 GE episodes/person-year in Italy, and 1.4 GE episodes/person-year in Denmark [15, 16, 18]. The incidence was, however, substantially higher than estimates from France, with 0.3 GE episodes/person-year during 2009–2010 [20]. In the latter study authors attributed the low estimate to a higher exclusion rate of GE episodes likely due to non-infectious causes.

Table 5. Average costs of gastroenteritis by resource unit and age group, and estimated total costs for the Netherlands

Average costs per GE case (€)	0 – 4	5 – 17	18 – 24	25 – 44	45 – 64	65+	All ages
Direct healthcare costs							
GP visit	6.22	2.68	3.28	5.19	4.79	10.85	5.18
Hospitalization	46.21	21.33	0.00	24.72	60.81	403.52	68.45
Medicine prescription	1.31	0.14	0.35	0.64	0.75	1.21	0.69
Stool sample laboratory test	1.94	0.89	0.41	0.00	2.56	14.55	0.98
Total	55.68	25.04	4.04	30.55	68.91	430.14	80.80
Patient costs							
Car/bus transport	0.30	0.14	0.13	0.24	0.25	0.58	0.24
Ambulance transport	0.16	0.07	0.00	0.61	1.50	30.00	3.59
Medicine OCT	0.40	0.56	0.60	0.60	0.68	0.49	0.58
Total	0.86	0.77	0.73	1.46	2.43	31.07	4.40
Productivity losses							
Absence paid work[a]	69.50	34.75	77.33	149.83	189.97	13.90	105.10
Average costs per GE episode	126.04	60.56	82.10	181.83	261.31	475.11	190.70
Total costs Dutch population							
Population size	872,500	2,532,390	1,479,740	4,215,470	4,824,970	3,278,160	17,203,230
Total annual costs (millions)	77.28	44.63	64.18	231.88	270.06	256.52	944.56
Average annual costs per person	88.57	17.62	43.37	55.01	55.97	78.25	54.91
With children in the household	88.57	17.62	49.55	59.51	42.80	n/a	53.38[b]
Without children in the household	n/a	n/a	41.09	50.88	61.09	78.25	54.85[b]

GE: gastroenteritis, GP: general practitioner, OCT: over-the-counter

[a]Of themselves or a caregiver; [b]Only persons 18–64 years living with and without children in the household

Indeed, they excluded 46% of GE episodes, compared with 15% in our study. Of note, data collection in these studies were performed through telephone interviews, while we performed a web-based survey. The major advantage of a web-based survey is that it is less time-consuming, but a compromise is the response rate, which was 20% in our survey compared to 26–81% in the telephone surveys, which might affect the representativeness of our sample [15, 16, 18, 20]. Moreover, while our design required participants to have a computer with internet access, and a basic level of computer skills, telephone surveys require persons to have a landline and/or a registered mobile phone number in national phonebooks (unless they can be obtained from mobile network providers). Hence, both study designs might reach different populations. Although comparison of incidence estimates of community-acquired GE by other countries is hampered by the fact that they used different case definitions, estimates varied between 0.4 and 1.4 episodes/person-year: 0.4 episodes/person-year in Malta in 2004–2005, 0.6 episodes/person-year in Ireland in 2000–2001, 0.9 episodes/person-year in Australia in 2001–2002 1.2 episodes/person-year in Norway in 1999–2000, 1.2 episodes/person-year in Canada in 2005–2006, and 1.4 episodes/person-year in the United States of America in 1996–1997 [17, 19, 30-34].

The observed incidence of 0.81 episodes/person-year is likely an overestimation of the true incidence due to the retrospective nature of the study, which is prone to respondents attributing GE episodes that occurred earlier to the survey period (i.e. 'telescoping'), as well as selection bias of persons who recently experienced GE. Indeed, prospective studies tend to produce lower GE estimates, as was observed in a prospective population-based cohort studies from the Netherlands in 1999 and the United Kingdom in 2009, with 0.28 episodes/person-year and 0.27 episodes/person-year, respectively [7, 21]. In the latter study, they found a three-fold higher GE estimate in a retrospective study that was conducted in parallel to the prospective study in the same study population [21]. When applying the same correction factor to our GE incidence estimate, we would obtain an incidence of 0.29 episodes/person-year. This would suggest that the incidence of GE had remained at the same level as 20 years ago. Importantly, also prospective studies are subject to biases, including selection bias [22]. Hence, although the corrected incidence may provide an approximation of the true incidence, it should be interpreted with caution.

In children aged 0–4 years, living in a household with other children of less than 1 year of age was a risk factor for GE. This is most likely due to increased transmission between siblings [9, 10]. Interestingly, asthmatic children in this age group also had more GE, which is in line with findings from the previous study on GE in the Dutch community [2]. The hypothesis of asthmatic individuals being

generally frailer and thereby also more sensitive to GE has been proposed before, but the mechanism remains largely unclear [35, 36]. In children aged 5–17 years, those with an immune disorder were more likely to have GE. Indeed, immune-related diseases are associated with altered regulatory mechanisms between active immunity and tolerance in the gut, rendering these children more vulnerable to gastrointestinal infections [37].

In persons aged 18–24 and 25–44 years, having children aged 3–12 years in the household was also a risk factor for GE, especially if these children attended out-of-home care, likely reflecting transmission from young children to their parents [9, 10]. Interestingly, having children in the household was not a risk factor in persons aged 45–64 years. Indeed, these children were generally older and required less hands-on care, thereby likely reducing opportunities for transmission. A previous Dutch study among families with preschool children also found that GE risk of parents decreased with parents' age [9]. In the age group 25–44 years, non-native Dutch males more often had GE. Although we do not have a clear explanation, a community-based study in Italy found a similar higher GE incidence among non-Italian citizens [16]. Intermediate and highly educated persons in the age groups of 25–44 years and 45–64 years who were employed had GE less often than unemployed persons in the same age group. We hypothesize two possible reasons. First, the unemployed may reflect a population that is more vulnerable to infection, as health complaints might be the reason for unemployment, and second, they might be unemployed to take care of their children, which in itself could be a risk factor for GE. In contrast, being employed was a risk factor for GE in the age group of ≥65 years, which we believe reflects the 'younger elders' that are somewhat more socially active among the elderly. Indeed, three out of four cases with GE cases in this age group were 65–69 years. In the age group of ≥65 years, females more often had GE compared with native Dutch males. Although gender differences have been reported by other countries as well, albeit not always significant, they generally observed younger adult females to be at increased risk for GE, but not females aged ≥65 years [15, 17, 18]. Our finding is in agreement with a survey from the Netherlands, however, reporting that 26% of woman help caring for their grandchildren compared with 20% in males [38]. Furthermore, food allergy was a risk factor for GE in those 65+ years, for which the explanation could be bi-directional. While gastrointestinal infections have been identified as risk factor for food allergy, food allergy can reversely be the cause of diarrhea [39]. Moreover, specific changes to the immune system in some immune deficiency diseases may increase the risk of the developing food allergies, acting as a risk factor for GE [39].

We estimated the average costs of a GE episode in the Netherlands to be €191, which gives a total of €945 million costs for GE in the whole country in 2017 (= €55 per inhabitant). This is higher than previous estimates of €133–151 per GE episode (€150–170 after correcting for inflation) amounting to €611–695 million (€687–782 after correction) of total costs (€37–42 per inhabitant; €42–47 after correction) in 2009 [3]. This increase is partially attributable to increased healthcare costs. For example, costs for GP practice visits and hospitalization have increased by 18% and 7%, respectively, between 2009 and 2017. However, increased costs are mainly due to a higher hospitalization rate of GE cases in our study: 1.9% *vs*. 0.3% reported previously [3, 4],. It might be that our hospitalization rate is an overestimation due to selection bias of those who were recently hospitalized. The previous estimate was based on data from a prospective study on GE, which is less prone to this type of bias. Comparing our results with COI studies on GE from other countries is difficult due to differences in healthcare systems [17, 32, 40, 41]. However, in line with our findings, most studies report the major factor determining the magnitude of estimated economic costs to be productivity losses by the sick and/or their caregivers.

In contrast to the previous study estimating the GE incidence in the Dutch community in 2009, we could not exclude cases with vomiting due to regurgitation, motion sickness/vertigo, nauseous event, traumatic event, pregnancy, or alcohol/drug abuse, because these data were not available. Of the GE cases in our study, 13% had diarrhea and vomiting, 47% had diarrhea without vomiting, and 40% had vomiting without diarrhea. This differs from the 2009 study in the Netherlands, where 19% had both, 55% only diarrhea, and 26% only vomiting. The proportion of excluded GE episodes due to likely non-infectious causes in our study (15%) was similar to the one of the 2009 study (18%) [2]. Although some misclassification is likely to have occurred, this cannot fully explain the difference. A large proportion (43%) of GE cases reporting vomiting only, were children younger than 15 years, which is unlikely to be the result of alcohol abuse or pregnancy. Moreover, we have no indication for misclassification of vomiting due to regurgitation. In the age group 0–4 years, where regurgitation is most likely to occur, the incidence was even lower in our study than the previous one, i.e., 1.96 episodes/person-years compared with 2.89 episodes/person-year, respectively. This could be due to the exceptionally low rotavirus activity observed during 2014–2016 in the Netherlands, in the absence of rotavirus vaccination as part of the national immunization programme, which is the most important cause of GE in children in this age group [42]. The annual

number of laboratory confirmed rotavirus detections in the Netherlands ranged from 607 to 1323 during 2014–2016, compared with1936 rotavirus detections in 2009. Of note, rotavirus vaccination is scheduled to be implemented as part of the immunization programme in the Netherlands in 2019, but only for children with medical risk conditions predisposing to severe or complicated rotavirus GE, including prematurity, low birth weight, and severe congenital pathology [43, 44].

Several additional limitations should be recognised. The survey did not collect data on all parameters that were needed to calculate costs. Hence, we relied on existing data from the available literature to base our assumptions on. For example, we based the average duration of hospitalization on a study from 2009, while the current duration is likely to be shorter due to ongoing changes in Dutch hospital policy. Also, in order to ensure comparability with the previous study on GE costs in the Netherlands, we did not stratify productivity losses by mild, moderate (GP visits) and severe (hospitalized) cases. This could have led to an underestimation of the costs, since the hospitalization rate in our study was higher, which is associated with higher productivity losses. Moreover, it is crucial to consider the characteristics of respondents *vs*. those that did not participate. We adjusted (statistical weighting) for deviations of the sample from the underlying population it was drawn from, including an adjustment of the number of GP visits in our survey based on independently recorded GP surveillance data. Yet, our estimates represent the lower boundary of the true value of costs due to GE. First, we had no information (and could not therefore include data) on premature mortality and chronic sequelae due to GE, while previous research has shown that these generally dominate the estimates of costs associated with GE morbidity [24]. Second, we had no information on costs associated with productivity losses due to non-paid employment work (e.g. child care, voluntary work). And third, we considered only the direct effect of GE, and ignored potential sequelae triggered by GE infections [24, 28].

In conclusion, GE still poses a significant burden in the community, particularly in children of 0–4 years of age and in adults of ≤44 years of age living in households with children, and especially if children attend out-of-home childcare services. Similar to other industrialized countries, the major factor driving the costs due to GE was the loss of productivity (paid worktime) for the ill or the caregiver(s) of the ill. This study also provides new baseline GE incidence rates and characterizes the population at greatest risk. Moreover, the estimated annuals costs of GE can be used by public health policy makers to contextualize the economic burden of the disease in support of decision making.

Supplementary material

S1 Table. Unit costs in Euros, 2017

	Unit cost (€)	Source
Direct Healthcare costs		
General practitioner (per consultation)		
Visit	33.78	[6]
House visit	51.18	[6]
Phone call	17.40	[6]
Weighted[a]	52.94	[6, 25]
Hospitalization adults (19+ years) per day	487.23	[6]
Hospitalization children (0-18 years) per day	641.79	[6]
Medicine prescribed by a doctor	7.92	[6, 26]
Sample collection + testing	75.38	[4, 6]
Ambulance emergency transport	627.46	[6]
Patient costs		
Medicine over-the-counter	1.37	[26]
Travel costs per km (car/bus)	0.19	[6]
Parking fee (car)	3.07	[6]
Productivity losses		
Productivity losses from paid work[b]		
15 – 19 years	9.60	[6, 27]
20 – 24 years	18.65	[6, 27]
25 – 29 years	26.47	[6, 27]
30 – 34 years	32.64	[6, 27]
35 – 39 years	37.58	[6, 27]
40 – 44 years	40.72	[6, 27]
45 – 49 years	41.90	[6, 27]
50 – 54 years	42.32	[6, 27]
55 – 59 years	42.55	[6, 27]
60 – 64 years	42.00	[6, 27]
65+ years	36.41	[6, 27]

[a]Weighted cost for general practitioner (GP) visit (90% of cases), GP house visit (10% of cases) and GP telephone consultation (97% of cases); [b]Derived based on the average person productivity losses as given by Case Institute Netherlands (in Dutch: Zorginstituut Nederland) [6] and age-specific hour earnings as published by Statistic Netherlands [45].

S2 Table. Population characteristics of the ESBLAT study population (before and after weighting) and the Dutch population.

	The Netherlands[a] n=17,203,230		**Study population** n=9,512			**Study population standardized**[c] N=9,512		
	N	**%**	**N**	**%**	**Difference**[b]	**N**	**%**	Difference[c]
Age (years)								
0-4	872,500	5.1	536	5.6	+0.5	486	5.1	0.0
5-12	1,503,600	8.7	865	9.1	+0.4	858	9.0	+0.3
13-19	1,442,000	8.4	791	8.3	-0.1	783	8.2	-0.2
20-39	4,207,750	24.5	1,688	17.8	-6.7	2,344	24.6	+0.1
40-64	5,899,220	34.3	3,698	38.9	+4.6	3,286	34.6	+0.3
65-79	2,396,070	13.9	1,658	17.4	+3.5	1,335	14.0	+0.1
80+	882,090	5.1	276	2.9	-2.2	420	4.4	-0.7
Gender (female)	8,673,300	50.4	5,004	52.6	+2.2	4,786	50.3	-0.1
Location of residence[d]								
Urban	3,893,450	22.6	588	6.2	-16.4	2,152	22.6	0.0
Intermediate	10,513,460	61.1	5,914	62.2	+1.1	5,814	61.1	0.0
Rural	2,796,100	16.3	3,010	31.6	+15.3	1,547	16.3	0.0

[a]Population on 1 January 2016; [b]Difference between the study population and the population in the Netherlands; [c]Standardized by age (five-year age groups), gender and urbanization (urban, intermediate, rural); [d]Urban: ≥2500 addresses/km^2, intermediate: 500-2500 addresses/km^2, rural: <500 addresses/km^2

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CHAPTER 3

Societal burden and correlates of acute gastroenteritis in families with preschool children

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Published in Scientific Reports, 2016

Abstract

Background

Gastrointestinal infection morbidity remains high amongst preschool children in developed countries. We investigated the societal burden (incidence, healthcare utilization, and productivity loss) and correlates of acute gastroenteritis (AGE) in families with preschoolers.

Methods

Monthly for 25 months, 2000 families reported AGE symptoms and related care, productivity loss, and risk exposures for one preschooler and one parent.

Results

Amongst 8768 child-parent pairs enrolled, 7.3% parents and 17.4% children experienced AGE (0.95 episodes/parent-year and 2.25 episodes/child-year). Healthcare utilization was 18.3% (children) and 8.6% (parents), with 1.6% children hospitalized. Work absenteeism was 55.6% (median 1.5 days) and day-care absenteeism was 26.2% (median 1 day). Besides chronic enteropathies, antacid use, non-breastfeeding, and toddling age, risk factors for childhood AGE were having developmental disabilities, parental occupation in healthcare, multiple siblings, single-parent families, and ≤12-month day-care attendance. Risk factors for parental AGE were female gender, having multiple or developmentally-disabled day-care-attending children, antimicrobial use, and poor food-handling practices. Parents of AGE-affected children had a concurrent 4-fold increased AGE risk.

Conclusions

We concluded that AGE-causing agents spread widely in families with preschool children, causing high healthcare-seeking behaviours and productivity losses. Modifiable risk factors provide targets for AGE-reducing initiatives. Children may acquire some immunity to AGE after one year of day-care attendance.

Introduction

Acute gastroenteritis (AGE) is caused by a variety of infectious agents and some non-infectious conditions, often presenting with diarrhoea and/or vomiting that may impair daily functioning. AGE is usually self-limiting unless complicated by dehydration and extraintestinal manifestations. Although AGE mortality is low in developed countries and is decreasing globally[1], its morbidity remains high, particularly amongst preschool children during wintertime[2,3]. A naïve adaptive immune system and imperfect hygiene behaviours make children prone to gastrointestinal infections. Moreover, children attending day-care centres (DCCs), where the intensity of contacts amongst peers is considerable and the exposure to circulating pathogens is high[4], are at approximately twice the risk for infectious AGE as those home-cared[5,6]. Moreover, children may spread AGE-causing agents within the household, with child-to-parent transmission occurring approximately once every three AGE episodes in children[7], and with young children having a 3- to 8-fold increased risk for secondary AGE than adults[8].

Several studies on the burden of AGE have been published in Europe[2,3,5,9-18], the Americas[8,12,19-21], Asia and Oceania[12,22-25]. They focused either on the whole community[3,9,10,15-22,24,26] or on specific age groups, such as (primarily preschool) children[2,5-7,14,27,28], adults and elderly[11,23]. Amongst those studies from developed countries using a comparable case definition, the monthly community incidence of AGE was 2.6–11.1%, corresponding to 0.3–1.5 episodes/person-year. No study, however, focused specifically on the burden of AGE in families with preschool children and collected paired data for preschool children and their parents, though virtually all studies highlighted an increased risk for AGE in children[3,9,10,15-22,24,26], especially those attending DCCs[5,14,25,27], and in women[3,10,12,15,17,19-22,24]. It has been suggested that the still high morbidity of childhood AGE in developed countries may have a significant societal impact as a result of increased expenditure for medical care, alternative care (e.g. babysitting), and productivity losses due to worktime lost[5,7,14,29], especially given the increasing number of dual-income and single-parent families.

As families with preschool children are likely to account for a substantial portion of the AGE community burden, characterizing infection risks within the household is relevant to the public health endeavour. However, only a few studies have looked at more risk factors for childhood AGE than only general demographic characteristics[8,14,28], but they focused on DCC attendees[28], or subsets of children with

severe symptoms requiring medical care[8,14], which may not be entirely representative of AGE in the general population.

We performed a nationwide survey of families with preschool children to determine the societal burden and correlates of AGE in these children and their parents, including whether parents of AGE-affected children had an increased AGE risk. Our goal was to provide an evidence base for how and to what extent AGE in developed countries poses a burden on the family, on the healthcare system, and on the society as a whole, identifying also potential risk factors.

Methods

Study design

We performed a retrospective, monthly-repeated cross-sectional survey of families with <4-year-old children conducted in the Netherlands during October 2012–October 2014 (25 months), the so-called "Family & Health" survey. The focus on <4-year-old children is because at four years children become eligible to enrol in primary school in the Netherlands. Households were randomly selected from population registries of 335/415 municipalities covering 78% of the Dutch population (~16.8 million, ~5% aged <4 years).

Monthly, a random sample of 2000 <4-year-old children living in different households was drawn and their parents were invited by regular mail to complete a web-based questionnaire for the sampled child and for one parent chosen freely (Fig. 1). Sample size was based on 5% α-level, 1% precision, 22% expected AGE monthly incidence in <4-year-old children[3], a population of 653,165 <4-year-old children in the participating municipalities as of 1st June 2012, and an expected response rate of 13%, i.e. half of what can be expected from similar paper-and-pencil surveys[3]. The parent was informed on the main purpose of the survey, which was not limited to AGE, but generally aimed to assess the 'health status' of families with young children. Indeed, also other health conditions and aspects of family living standards were surveyed to complement data from current disease surveillance systems and population censuses. This study focused specifically on AGE given the still very high incidence of gastrointestinal infections in early childhood, their essentially preventable nature, and the limited published data on paediatric AGE outside the medical setting in developed countries. Upon completion of the questionnaire,

parents gave consent also on behalf of the child. A given household was sampled only once, so only one child-parent pair per household participated.

The questionnaire was developed by expanding those used in previous population-based studies in the Netherlands[3,28,30-32]. Questions covered the prior four weeks and focused on household characteristics, DCC attendance, chronic diseases, medications, symptoms, medical care, absenteeism from work and from DCC, occupation, contact with animals, leisure activities, and eating habits. The urbanization degree and socio-economic status (SES) at the postcode level were obtained from Statistics Netherlands (http://www.cbs.nl/en-GB/menu/home/default.htm).

Case definition

We used a standard AGE case definition based on the symptoms reported in the questionnaire[33]. An AGE case was defined as a person with ≥3 diarrhoeal discharges or any 'clinically relevant' vomiting (in absence of pregnancy) in 24 hours during the previous four weeks, but excluding those cases of probable non-infectious origin, i.e. with underlying enteropathies. By 'clinically relevant' vomiting we refer to vomiting events other than regurgitation, vomiting due to motion sickness/vertigo, traumatic event, nauseous event (e.g. seeing others vomit), or drug/alcohol abuse. The exclusion criteria of pregnancy and underlying enteropathy, however, were not applied for the risk factor analysis, as these factors were always controlled for in the analysis (see below). Multiple episodes of AGE during the 4-week recall period were not differentiated and counted as one.

Data analysis

The societal burden of AGE was expressed as incidence, episodes/person-year (calculated as [365/28] × [4-weekly incidence proportion])[3,5,32], medical care (general practitioner [GP] visits and hospitalizations), work and DCC days missed.

Logistic regression was used to identify factors associated with childhood and parental AGE. A total of 76 and 87 factors putatively associated with AGE in children and parents was assessed in a 'single-variable' analysis including the following control covariates: child age group (infants, ≤12 months; toddlers, 13–36 months; pre-schoolers, 37–47 months) or parent age group (≤30, 31–34, 35–37, ≥38 years), gender, pregnancy, SES (low, intermediate, high), urbanization degree (<500, 500–1000, 1000–1500, 1500–2500, >2500 addresses/km^2), season (Autumn, September–November; winter, December–February; spring, March–May; summer,

June–August), year (2012–2014), and underlying enteropathies (e.g. bowel cancer, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis, celiac disease, Crohn's disease, food allergy/intolerance, malabsorption syndromes, gastroesophageal reflux disease, chronic gastritis, and peptic ulcer disease). Variables with a $p \leq 0.10$ were selected for inclusion in a multivariable model built in backward stepwise fashion, with variables showing a $p < 0.05$ being retained in the final model; the above covariates were always controlled for. The effect of removing variables on the other covariates was also monitored. Biologically plausible interactions were also tested as reported elsewhere[30,31]. Associations were expressed as risk ratios (RR) providing 95% confidence intervals (95%CI). Selection between collinear variables was made based on improved model fit as revealed by the Akaike information criterion (AIC). A complete record analysis was performed. Selection of covariates was theoretically informed, and variables to be tested were chosen based on previous studies, biological plausibility of being associated with AGE, and scientific interest of the research team. The variable 'presence/absence of AGE in the participating child' was then included as an additional explanatory variable in the multivariable model predicting parental AGE, allowing the association between AGE in the participating children and (their) parents to be tested. However, as it would not have been entirely correct to explain parental AGE with children's AGE since we had no information on the AGE status of their non-participating siblings and other family members, risk factors for parental AGE were studied independently of children's AGE. The final multivariable models showed an overall statistical significance (likelihood-ratio χ^2-test, $p < 0.05$) and goodness-of-fit (Hosmer-Lemeshow test, $p > 0.05$). To cross-validate the inferences of the fitted models, bias-corrected bootstrap estimates were also calculated (1000 replications) and compared with the standard ones[34]. As these confidence intervals did not differ significantly, the standard ones were reported. For simplicity, only the final multivariable model results were presented. Statistical analyses were performed using STATA 13 (StataCorp LP, College Station, TX, USA).

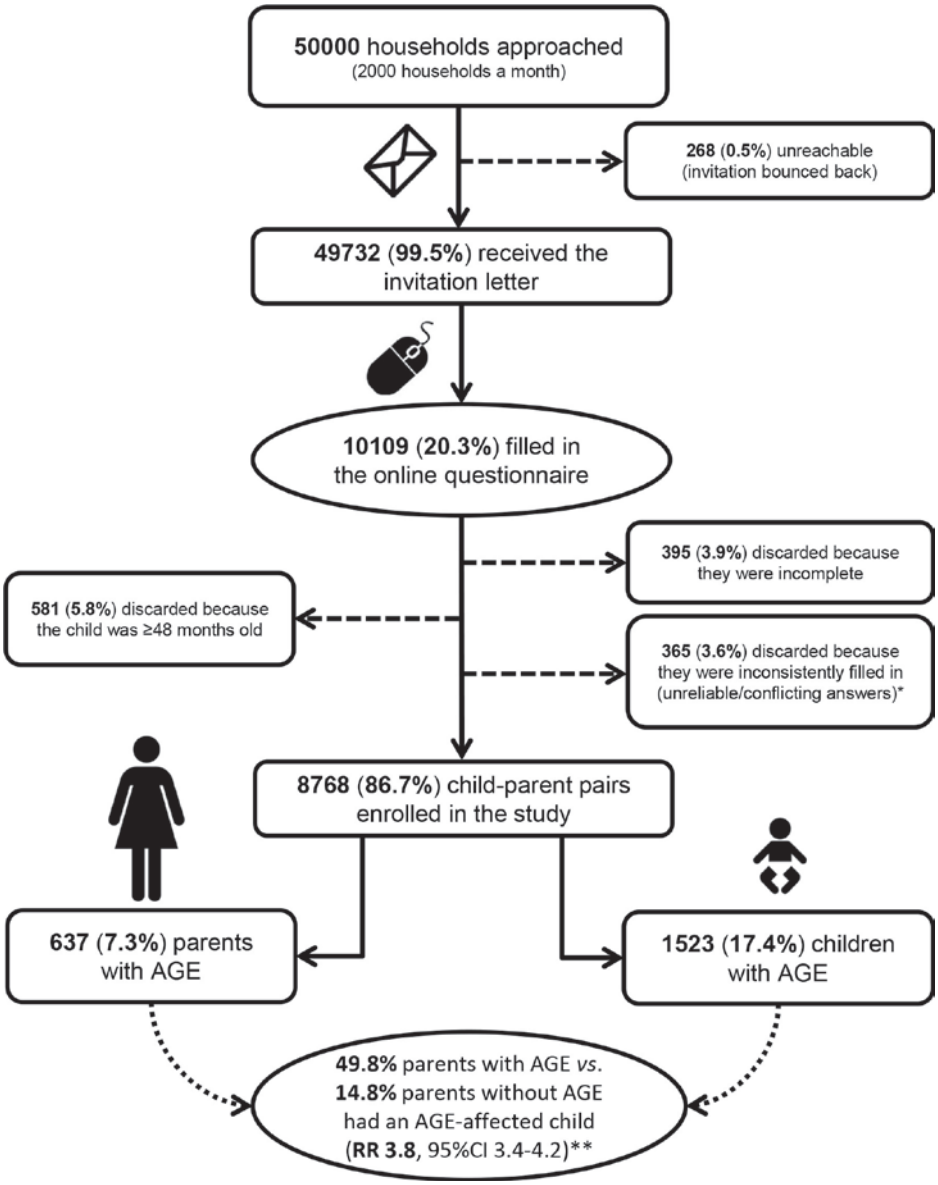

Figure 1. Flowchart of the sampling design. AGE = acute gastroenteritis; RR = risk ratio; 95%CI = 95% confidence interval. *The most common unreliable/conflicting answers leading to the questionnaire being discarded were those denoting mixing up of answers for the child with those for the parent and vice versa, or when the parents had reported data for a child other than the one invited to participate. Other examples of unreliable data were, for instance, reporting to be male and pregnant, being too young to be a parent (e.g. 10 year old), evident mistakes in reporting the date of birth (e.g. being born in the 1800s), reporting to have a partner living in the household but that only 1 adult lived in the household, reporting that no children lived in the household, etc. **Adjusted for the variables presented in Table 3.

Results

Sample characteristics

Overall, 10109 (20.3%) of the 49732 households receiving the invitation completed the questionnaire; 268 (0.5%) were unreachable (incomplete/changed address). After data cleaning (illustrated in Fig. 1), 8768 child-parent pairs were enrolled in the study. With regard to child's age and gender, season, SES, and urbanization degree, for which we had information for both participants and non-participants, our child-parent pairs were representative of the target sample for child's age, gender, and season, but there were significant differences for SES and urbanization degree. Specifically, the survey tended to underrepresent households from highly urbanized areas and from areas with high SES, and to overrepresent those from lowly urbanized/rural areas and from areas with low SES. To address this, we applied a weighting adjustment by SES and urbanization degree so that the incidence estimations accounted for imperfect representativity of the sample by weighting it back to the population from which it was drawn.

Questionnaires were completed by 7268 mothers (median age 34 years, interquartile range [IQR] 31–37) and 1500 fathers (37, 33–41), matching with 4210 female (median age 27 months, IQR 16–37) and 4558 male (26, 16–38) children. DCCs were attended by 50.4% of the children for a median of two days/week (IQR 2–4). Median duration of DCC attendance was 24 months (IQR 9–24). Most families (98.0%) had two parents, and had two children (47.2%), followed by those with one child (35.0%) and ≥3 children (17.8%).

Burden

A total of 637 (7.3%) parents and 1523 (17.4%) children experienced AGE, corresponding to a (weighted) incidence of 0.95 episodes/parent-year (95%CI 0.88–1.02) and 2.25 episodes/child-year (2.15–2.36). In 317 (3.6%) child-parent pairs, both parent and child experienced AGE in the same 4-week period. Since our sample overrepresented mothers relative to fathers, sex-standardized parental incidence was also calculated: 6.5%, i.e. 0.85 episodes/parent-year (95%CI 0.77–0.94).

Monthly AGE incidence varied from 3.5–12.9% in parents to 11.0–26.3% in children, peaking in winter and decreasing in summer (Fig. 2). Of the 637 parent-cases, 22.8% had diarrhoea and vomiting, 47.7% only diarrhoea, and 29.1% only vomiting. Of the 1523 child-cases, 20.9% had diarrhoea and vomiting, 32.2% only diarrhoea, and 46.9% only vomiting; additional symptom descriptives are reported

in the Supplementary Table S1. AGE lasted for a median of three days in both parents (IQR 2–4) and children (1–5). GP utilization was 18.3% for child cases and 8.6% for parent cases, and 1.6% of child cases but no parents were hospitalized. Most hospitalized children were toddlers (54.2%) and infants (37.5%), and attended DCCs (64.3%). Absenteeism from work was reported by 29.8% of parent cases and (by the parents of) 15.8% of child cases. Additionally, for 7.2% and 2.8% of parent and child cases, respectively, other people than the parents had to miss work. Child DCC absenteeism was 26.2% (Table 1).

Table 1. Burden of acute gastroenteritis in parents and children.

	Parents with AGE (*n*=637)	**Children with AGE** (*n*=1523)
Contacted the general practitioner	55 (8.6%)	278 (18.3%)
Hospitalized	None	25 (1.6%)
Parent(s) absent from work	190 (29.8%)	241 (15.8%)
median days of absence (IQR)	1.5 (1–2)	1 (1–2)
Others than the parent(s) absent from work	46 (7.2%)	42 (2.8%)
median days of absence (IQR)	1 (1–1)	1 (1–1)
Child absent from day-care	-	222/847* (26.2%)
median days of absence (IQR)	-	1 (1–2)
Median duration of illness in days (IQR)	3 (2–4)	3 (1–5)
Medication use**	100 (15.7%)	135 (8.9%)

IQR = Interquartile range; AGE = acute gastroenteritis

*Number of AGE child cases attending day-care centres; **Antidiarrhoeals, antiemetics, antimicrobials, antispasmodic, antipyretic, and anti-inflammatory drugs.

Risk factors

Significant risk factors for childhood AGE in the final multivariable model including 1536 AGE-affected and 7232 AGE-free children are reported in Table 2. These were having underlying gastrointestinal, respiratory, or developmental conditions, using gastric antacids, having the participating parent employed in healthcare, living in a single-parent family, and having multiple siblings. Compared to home-cared children, those attending DCCs were at increased risk of AGE until twelve months of attendance, but afterwards this risk was not significant. Several animals in/around the households were tested for association with AGE, but only ownership of poultry/birds was significant. There was an interaction between child age and breastfeeding: breastfed infants of ≤6 months of age (but not those aged >6 months)

had a significantly lower AGE risk than the never-breastfed infants, against which toddlers had an increased AGE risk (Table 2).

Table 3 shows the factors independently associated with parental AGE in the final multivariable model based on 764 AGE-affected and 8004 AGE-free parents. These were female gender, having underlying enteropathies, using gastric antacids, having a DCC-attending developmentally-disabled child, having multiple DCC-attending children, using antimicrobials in autumn/winter for pre-existing conditions other than AGE, primary consumption of meat bought directly from farmers, and having to normally wait for >1 hour between grocery shopping and food refrigeration. Older age and cleaning the fridge weekly were protective (Table 3). Parents whose participating children had AGE were at increased risk for AGI (RR 3.77, 95%CI 3.38–4.19).

Discussion

AGE was estimated at 2.26 episodes/child-year and 0.95 episodes/parent-year, in line with the last Dutch community-based survey (2009–2010) employing a comparable case definition[3]. However, we found higher rates of GP visits (18.6% for children and 8.6% for parents) and hospitalization (1.6%) compared to those observed previously (GP visits: 12.8% for 'children', *n*= 39, and 5.7% for 'adults', *n*= 105; hospitalization: 0.7%, *n*= 146)[3]. An explanation may be the longer duration of our AGE episodes, as 50% of all our cases *vs*. 29% (*n*= 133) of the previous ones had symptoms for ≥3 days (Table 1) and were therefore likely to contact a physician[3,10,12]. This suggests a higher severity of AGE in families with young children, as also supported by the higher absenteeism from work (37% for parental AGE and 18.6% for childhood AGE in our survey *vs*. 14.4% among all 146 AGE cases found previously) and from DCC (26% *vs*. 13.7%)[3].

AGE seasonality was similar between children and parents (Fig. 2), whereas in surveys that did not specifically focus on parents, childhood AGE seasonality differed considerably from that of the rest of the (adult) population[9,10], as it mainly reflected the age-specific pathogen distribution in children and adults throughout the year[35,36]. Our finding is therefore suggestive of common aetiological agents, particularly viruses, shaping the observed seasonal patterns, indicating either shared sources of infection or household transmission. The strong association between AGE occurrence in children and (their) parents supports this hypothesis.

Table 2. Multivariable risk ratios, corresponding 95% confidence intervals and adjusted predictions of the factors significantly associated with acute gastroenteritis in children of <4 years of age.

	Children with AGE (*n* = 1536)	Children without AGE (*n* = 7232)	Adjusted RR[1] (95%CI)	Adjusted AGE predictions[1,7]
Season[2]				
Summer	283 (18.42%)	1765 (24.41%)	Reference	13.56%
Spring	410 (26.69%)	1899 (26.26%)	1.28 (1.12-1.45)	17.44%
Autumn	396 (25.78%)	2066 (28.57%)	1.21 (1.05-1.39)	16.53%
Winter	447 (29.10%)	1502 (20.77%)	1.67 (1.48-1.88)	23.08%
Child age[3] x breastfeeding				
Never-breastfed infant	64 (4.17%)	352 (4.87%)	Reference	14.08%
≤6 month-old breastfed infant	19 (1.24%)	245 (3.39%)	0.51 (0.31-0.84)	7.22%
>6 month-old breastfed infant	53 (3.45%)	232 (3.21%)	1.29 (0.92-1.74)	18.21%
Infant with unknown breastfeeding history	130 (8.46%)	531 (7.34%)	1.31 (0.99-1.69)	18.57%
Toddler	913 (59.44%)	3886 (53.73%)	1.34 (1.06-1.66)	18.93%
Pre-schooler	357 (23.24%)	1986 (27.46%)	1.13 (0.87-1.44)	15.96%
Chronic enteropathies[4]				
No	1352 (88.02%)	6786 (93.83%)	Reference	16.82%
Yes	184 (11.98%)	446 (6.17%)	1.55 (1.32-1.79)	25.80%
Chronic respiratory diseases[5]				
No	1388 (90.36%)	6853 (94.76%)	Reference	17.04%
Yes	148 (9.64%)	379 (5.24%)	1.44 (1.23-1.68)	24.33%
Developmental disabilities[6]				
No	1477 (96.16%)	7118 (98.42%)	Reference	17.26%
Yes	59 (3.84%)	114 (1.58%)	1.72 (1.35-2.15)	29.26%
Using gastric antacids				
No	1488 (96.88%)	7152 (98.89%)	Reference	17.35%
Yes	48 (3.13%)	80 (1.11%)	1.56 (1.13-2.09)	26.77%

Table 2. Continued.

	Children with AGE (*n* = 1536)	Children without AGE (*n* = 7232)	Adjusted RR[1] (95%CI)	Adjusted AGE predictions[1,7]
Parent working in healthcare				
No	1148 (74.74%)	5611 (77.59%)	Reference	17.04%
Yes	388 (25.26%)	1621 (22.41%)	1.13 (1.01-1.25)	19.11%
Single-parent family				
No	1494 (97.27%)	7101 (98.19%)	Reference	17.40%
Yes	42 (2.73%)	131 (1.81%)	1.35 (1.01-1.76)	23.25%
***N* children in the house**				
1 child	492 (32.03%)	2574 (35.59%)	Reference	15.73%
2 children	770 (50.13%)	3370 (46.60%)	1.19 (1.07-1.32)	18.69%
≥3 children	274 (17.84%)	1288 (17.81%)	1.15 (1.00-1.31)	17.99%
Cumulated DCC attendance				
None	680 (44.27%)	3670 (50.75%)	Reference	15.79%
1-3 months	72 (4.69%)	275 (3.80%)	1.47 (1.18-1.81)	23.25%
4-6 months	83 (5.40%)	250 (3.46%)	1.51 (1.22-1.84)	23.86%
7-12 months	291 (18.95%)	1029 (14.23%)	1.33 (1.18-1.51)	21.04%
13-24 months	246 (16.02%)	1094 (15.13%)	1.11 (0.97-1.27)	17.56%
>24 months	164 (10.68%)	914 (12.64%)	1.03 (0.86-1.22)	16.20%
Owning poultry and/or birds				
No	1406 (91.54%)	6724 (92.98%)	Reference	17.25%
Yes	130 (8.46%)	508 (7.02%)	1.23 (1.04-1.44)	21.05%

AGE = acute gastroenteritis; RR = risk ratio; 95%CI = 95% confidence interval; DCC = day-care centre. [1]Adjusted for urbanization degree, socio-economic status, year, and child's gender in addition to all the other variables included in this table; [2]Autumn, September–November; winter, December–February; spring, March–May; summer, June–August; [3]Infant, ≤12 months; toddler, 13–36 months; pre-schooler, 37–47 months; [4]E.g. bowel cancer, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis, celiac disease, Crohn's disease, food allergy/intolerance, malabsorption syndromes, gastroesophageal reflux disease, chronic gastritis, and peptic ulcer disease; [5]E.g. asthma, chronic obstructive pulmonary disease and other chronic lung diseases, respiratory allergies, lung cancer, and pulmonary hypertension; [6]E.g. mental retardation, cerebral palsy, autism spectrum disorders, attention-deficit/hyperactivity disorder, Down's syndrome and other genetic disorders, congenital defects, learning disabilities, mental illness, and traumatic brain injury; [7]Aka predictive margins are the adjusted prevalences of AGE for each stratum of the independent variables included in the model and denote the probability for an AGE event to occur for individuals in those strata.

Table 3. Multivariable risk ratios, corresponding 95% confidence intervals and adjusted predictions of the factors significantly associated with acute gastroenteritis in parents of children of <4 years of age.

	Parents with AGE (*n* = 764)	Parents without AGE (*n* = 8004)	Adjusted RR[1] (95%CI)	Adjusted AGE predictions[1,5]
Season[2]				
Summer	134 (17.54%)	1914 (23.91%)	Reference	6.97%
Spring	198 (25.92%)	2111 (26.37%)	1.33 (1.08-1.63)	9.27%
Autumn	214 (28.01%)	2248 (28.09%)	1.13 (0.90-1.41)	7.88%
Winter	218 (28.53%)	1731 (21.63%)	1.57 (1.28-1.92)	10.93%
Parent age				
≤30 years	160 (20.94%)	1325 (16.55%)	Reference	11.07%
31-34 years	255 (33.38%)	2457 (30.70%)	0.84 (0.68-1.02)	9.35%
35-37 years	181 (23.69%)	1845 (23.05%)	0.77 (0.62-0.96)	8.69%
≥38 years	168 (21.99%)	2377 (29.70%)	0.59 (0.47-0.74)	6.68%
Parent gender				
Male	92 (12.04%)	1408 (17.59%)	Reference	6.56%
Female	672 (87.96%)	6596 (82.41%)	1.39 (1.13-1.7)	9.13%
Chronic enteropathies[3]				
No	672 (87.96%)	7627 (95.29%)	Reference	8.16%
Yes	92 (12.04%)	377 (4.71%)	2.18 (1.76-2.67)	17.56%
Using gastric antacids				
No	747 (97.77%)	7950 (99.33%)	Reference	8.64%
Yes	17 (2.23%)	54 (0.67%)	1.82 (1.08-2.94)	15.47%
Using antimicrobials for conditions other than AGE				
No	732 (95.81%)	7880 (98.45%)	Reference	8.55%
Yes, in autumn/winter	17 (2.23%)	53 (0.66%)	2.15 (1.31-3.36)	18.02%
Yes, in spring/summer	15 (1.96%)	71 (0.89%)	1.65 (0.97-2.69)	13.94%
***N* children in the house attending DCCs**				
None	296 (38.74%)	3640 (45.48%)	Reference	7.29%
1 child	285 (37.30%)	2944 (36.78%)	1.26 (1.07-1.47)	9.17%

Table 3. Continued.

	Parents with AGE (n = 764)	Parents without AGE (n = 8004)	Adjusted RR[1] (95%CI)	Adjusted AGE predictions[1,5]
2 children	168 (21.99%)	1325 (16.55%)	1.54 (1.28-1.85)	11.22%
≥3 children	15 (1.96%)	95 (1.19%)	1.95 (1.19-3.07)	14.18%
Having a child with developmental disabilities[4]				
No	738 (96.60%)	7857 (98.16%)	Reference	8.60%
Yes, attending a DCC	18 (2.36%)	60 (0.75%)	1.03 (0.51-1.97)	19.00%
Yes, not attending a DCC	8 (1.05%)	87 (1.09%)	2.27 (1.42-3.43)	8.84%
Primary type of meat consumed				
No meat consumption	26 (3.40%)	343 (4.29%)	Reference	7.88%
Regular meat from butcher/supermarket	629 (82.33%)	6572 (82.11%)	1.10 (0.75-1.58)	8.64%
Meat directly from farmers	32 (4.19%)	210 (2.62%)	1.69 (1.03-2.66)	13.20%
Organic meat	77 (10.08%)	879 (10.98%)	1.07 (0.70-1.62)	8.45%
Cleaning frequency of the fridge				
Less often than once a month	551 (72.12%)	5697 (71.18%)	Reference	8.88%
Every month	175 (23.04%)	1781 (22.25%)	1.00 (0.84-1.18)	8.89%
Every week	37 (4.84%)	526 (6.57%)	0.70 (0.50-0.98)	6.32%
Average time between grocery shopping and refrigeration				
<1 hour	429 (56.15%)	4841 (60.48%)	Reference	8.16%
1-2 hours	296 (38.74%)	2847 (35.57%)	1.15 (1.00-1.33)	9.39%
>2 hours	39 (5.10%)	316 (3.95%)	1.35 (0.98-1.83)	10.93%

AGE = acute gastroenteritis; RR = risk ratio; 95%CI = 95% confidence interval; DCC = day-care centre. [1]Adjusted for urbanization degree, socio-economic status, year, and pregnancy status in addition to all the other variables included in this table; [2]Autumn, September–November; winter, December–February; spring, March–May; summer, June–August; [3]E.g. bowel cancer, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis, celiac disease, Crohn's disease, food allergy/intolerance, malabsorption syndromes, gastroesophageal reflux disease, chronic gastritis, and peptic ulcer disease; [4]E.g. mental retardation, cerebral palsy, autism spectrum disorders, attention-deficit/hyperactivity disorder, Down's syndrome and other genetic disorders, congenital defects, learning disabilities, mental illness, and traumatic brain injury; [5]Aka predictive margins are the adjusted prevalences of AGE for each stratum of the independent variables included in the model and denote the probability for an AGE event to occur for individuals in those strata.

However, because the onset of illness in children and parents was not available, we could not determine whether household transmission occurred, nor if it had any directionality, though even this would not have provided conclusive evidence. Nevertheless, previous research showed that parents frequently acquire AGE in secondary transmission from their children[7,8]. Our finding that mothers were more likely to experience AGE than fathers supports this notion, as previous studies suggested that the traditionally female-oriented task of preparing food[15,37] and caring for the children[10,12,15] may place women at increased exposure to enteropathogens than men.

Some pathogens known to cause foremost respiratory infections may also cause AGE symptoms and vice versa[38,39]. When excluding AGE cases with concurrent respiratory symptoms (cough, sore throat, runny nose, or breathing difficulties), our AGE incidence decreased by 55.4% in children and 30.0% in parents (results not shown). Similar estimates were reported in other surveys[10,12,39], suggesting that respiratory pathogens may contribute to the AGE burden.

Alike all retrospective surveys, recall bias and specifically 'telescoping', i.e. when people remember disease episodes as being more recent than they actually are, may have occurred, leading to an overestimation of AGE incidence[26]. However, recall periods of ≥30 days may even lead to an underestimation of AGE incidence as respondents may forget (mild) illness episodes[13]. The somewhat low response rate (20.3%), although comparable to other studies[9,11,22], may have represented a potential source of bias, as people who suffered from AGE might have been more motivated to participate. However, response rates were not particularly high in winter when AGE incidence was highest (Figure 2). Finally, we cannot exclude that some misclassification of AGE cases due to non-infectious causes might have occurred.

Breastfeeding was confirmed to reduce AGE risk in the first six months of life compared to never-breastfed infants[40]. The increased risk for toddlers *vs.* infants is probably related to their changed feeding habits and start of active interactions with peers and surroundings. Having underlying enteropathies and taking gastric antacids, especially proton-pump inhibitors, have previously been associated with AGE[3] and specific bacterial infections like salmonellosis[31] and campylobacteriosis[30]. It is conceivable that a chronically disturbed digestive tract may facilitate infections and that neutralization of gastric acidity may favour a pathogen's survival during passage through the stomach. Increased AGE occurrence in asthmatic children has also been documented[3,41], though the reasons remain unclear.

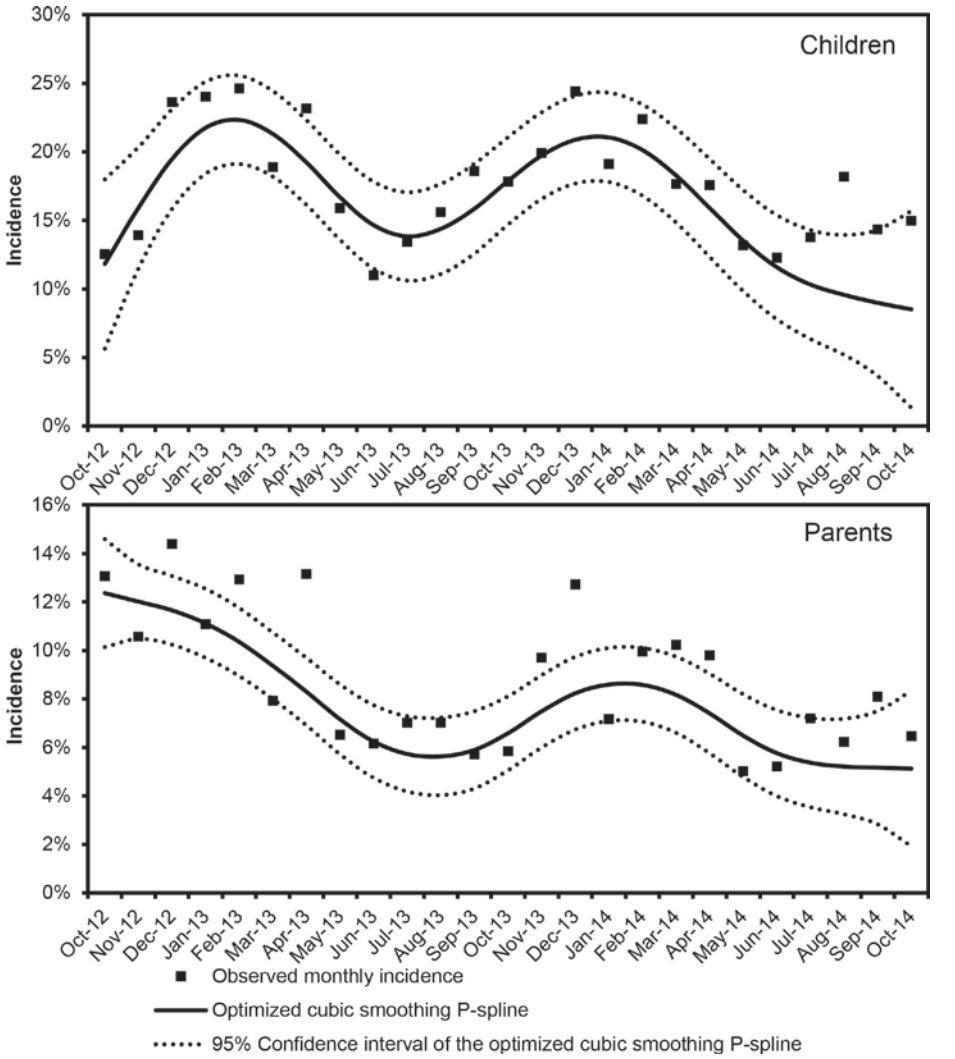

Figure 2. Incidence of acute gastroenteritis in children of <4 years of age and in their parents (*n*=8768 child-parent pairs) by study month. An optimized cubic smoothing P-spline function is fitted to the observed data. Autumn, September–November; winter, December–February; spring, March–May; summer, June–August.

We found that children with developmental disabilities had an increased AGE risk. Previous research showed that children with autism-spectrum disorders have an 8-fold increased risk for AGE than non-disabled children[42]. The underlying reasons might be that these children more often display at-risk behaviours, such as suboptimal hygienic habits and extreme food selectivity. Our study indicated that

also the parents of (DCC-attending) disabled children were at increased risk for AGE, possibly reflecting a higher chance of secondary transmission, especially since these children might need extra/special assistance for personal hygiene.

Parental occupation in healthcare was a risk factor for childhood AGE. Although this may suggest pathogen carriage from a parent's work setting to the household, it is also possible that parents employed in healthcare, by virtue of their knowledge on health-related topics, reported more thoroughly. Challenges in continual supervision of a child's hygiene behaviours and nurture needs due to likely parental task overload and recourse to various 'outsourced' childcare arrangements might explain the higher risk in children of single-parent families, while increased opportunities for household transmission would explain the higher risk associated with multiple siblings. Interestingly, of all the animals kept in/around the household we tested for, only poultry/birds were significant, warranting further attention on this exposure.

Compared to home-cared children, those attending DCCs were at increased risk of AGE for the first year of attendance, but not beyond, suggesting that immunological maturation may result from the (excess of) infections experienced during that first year of attendance (since we adjusted for child's age), as also suggested by a recent Danish study[43]. Prior exposure and increased immunological maturity in our parents (i.e. young adults) may also explain why older parents experienced less AGE.

Parents were at increased risk of AGE with increasing number of DCC-attending children, confirming that attending DCCs does not only pose a risk to children, but also to parents via increased secondary transmission[7]. Another risk factor for parental AGE was using antimicrobials in autumn/winter. Antimicrobial-induced disturbance of the gut microbiome in a period of high pathogen activity would reduce intrinsic resistance to clinically overt infections. While viral aetiology seems plausible given the seasons in question and that taking antibiotics has been reported to protect against salmonellosis[31] and campylobacteriosis[30], antimicrobial-mediated depletion of normal gut microbiota can also diminish enteric virus replication[44]. Yet, *Clostridium difficile* infections, which seasonal peak occurs during autumn/winter, have increasingly been related to antimicrobial use[45]. Lastly, we pointed out that buying meat directly from farmers, delays in food refrigeration, and infrequent fridge cleaning increase parental AGE risk.

In conclusion, AGE occurs frequently in families with preschool children, causing high healthcare-seeking behaviours and productivity losses. Similar seasonality, strong association between parental and childhood AGE, and increased maternal

risk suggest extensive household transmission of AGE-causing agents. The identified risk factors, some of which are in principle modifiable (i.e. food safety practices, medication use, breastfeeding, animal exposure), might provide targets for AGE-reducing initiatives in the surveyed population. These should focus on improving child-rearing hygiene, targeting mainly mothers, families with multiple siblings, with developmentally-disabled children, single parents, and with parents working in healthcare. A particular target should be DCCs, as they were a major determinant of AGE in both children and parents. However, the increased risk amongst DCC attendees lasted until one year of attendance, suggesting a gradual acquisition of immunity. This is a reassuring finding given the increased reliance on DCCs in high-income countries due to growing employment of women and rise in single-parent households. Efforts are nonetheless needed to improve hygiene in DCCs as to prevent pathogen transmission.

Supplementary material

Supplementary Table S1. Self-reported gastrointestinal symptoms in the child-parent pairs.

	Parents (*n*=8768)	**Children** (*n*=8768)
Vomiting	414 (4.7%)	1041 (11.9%)
Diarrhoea (≥3 diarrhoeal discharges in 24 h)	509 (5.8%)	820 (9.4%)
Nausea	610 (7.0%)	291 (3.3%)
Abdominal pain	870 (9.9%)	924 (10.5%)
Mucus in the stool	71 (0.8%)	135 (1.5%)
Blood in the stool	51 (0.6%)	28 (0.3%)
Discoloration in the stool	77 (0.9%)	286 (3.3%)
Acute gastrointestinal illness[1]	**Parents with AGE** (*n*=637)	**Children with AGE** (*n*=1523)
Vomiting	333 (52.3%)	1033 (67.8%)
Diarrhoea (≥3 diarrhoeal discharges in 24 h)	449 (70.5%)	809 (53.1%)
Nausea	285 (44.7%)	241 (15.8%)
Abdominal pain	301 (47.3%)	446 (29.3%)
Mucus in the stool	21 (3.3%)	90 (5.9%)
Blood in the stool	10 (1.6%)	13 (0.9%)
Discoloration in the stool	32 (5.0%)	165 (10.8%)

AGE = acute gastroenteritis; IQR = interquartile range

[1]Defined as having ≥3 diarrhoeal discharges and/or any vomiting (in absence of pregnancy) in 24 h, but excluding those with underlying gastrointestinal diseases that may have caused these symptoms.

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CHAPTER 4

Potential causative agents of acute gastroenteritis in households with preschool children: prevalence, risk factors, clinical relevance, and household transmission

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Published in European Journal of Clinical Microbiology & Infectious Diseases, 2016

Abstract

Background

Acute gastroenteritis (AGE) morbidity remains high amongst preschool children, posing a significant societal burden. Empirical data on AGE-causing agents is needed to gauge their clinical relevance and identify agent-specific targets for control. We assessed the prevalence, risk factors and association with symptoms for enteropathogens in households with preschool children.

Methods

A monthly-repeated cross-sectional survey of enteropathogens in households with preschool children was performed. A parent-child pair per household (*n*=907 households) provided faecal samples and reported their symptoms and potential risk exposures. Samples were tested by multiplex reverse transcription polymerase chain reaction (RT-PCR) for 19 enteropathogens. Associations were assessed using logistic regression.

Results

28.3% of children (*n*=981) and 15.6% of parents (*n*=971) carried pathogenic bacteria and/or *Escherichia coli*-associated pathogenicity genes, and 6.5% and 3.3% carried viruses, respectively. *Giardia lamblia* (4.6% of children, 2.5% of parents) and *Dientamoeba fragilis* (36%, 39%, respectively) were the main parasites, and were associated with pet exposure. Living in rural areas was associated with carriage of pathogenic *E. coli*, norovirus I, and *D. fragilis*. Pathogenic *E. coli* was associated with summertime and livestock exposure. Attending day-care centres increased the risk of carrying norovirus, sapovirus, and *G. lamblia*. Viruses occurred mainly in winter and were associated with AGE symptoms. Child-parent associations were found for bacterial pathogenicity genes, viruses, *G. lamblia* and *D. fragilis*.

Conclusions

Enteropathogens spread widely in households with preschool children, particularly viruses, which more often cause symptoms. While bacteria predominate during summer and in those exposed to livestock, viruses predominate in wintertime and, like *G. lamblia*, are widespread amongst day-care centre attendees.

Introduction

Although nowadays acute gastroenteritis (AGE) is hardly a life-threatening illness in high-income countries, it still poses a high morbidity (1), with an estimated community incidence rate of 0.96 episodes of gastroenteritis per person/year in a country like the Netherlands (2). Children of preschool age are particularly prone to AGE on account of their immunological naivety, immature hygiene behaviours, and frequent contacts with peers. Moreover, because children can shed enteropathogens for protracted periods before and after the symptomatic phase, there is plenty of opportunity for pathogen spread (3). Recent research showed that 78% of stool samples from children attending day-care centres (DCC) in the Netherlands were positive for at least one known enteropathogen, with 95% of these samples being obtained from children with no AGE symptoms (4). Studies with sampling designs that are not driven by symptomatology are of paramount importance to understand the clinical relevance of enteropathogens present in faecal samples.

A Canadian study has estimated that AGE transmission from DCC-attending children to their parents occurs about once every three AGE episodes in children (5). We have recently reported that parents (and particularly mothers) of AGE-affected children of preschool age in the Netherlands have a four-fold increased risk to experience AGE in the same month as their children, posing a significant societal burden (6). They also exhibit a very similar seasonal pattern, which is indicative of household transmission of AGE-causing agents (6). However, this study did not provide aetiological evidence for the potential causative agents of AGE (hereafter interchangeably referred to as enteropathogens), the knowledge of which is, therefore, still needed to substantiate these findings.

To identify targets for control as to reduce the burden of gastrointestinal infections in households with preschool children in a high-income country like the Netherlands, this study sought to determine which enteropathogens circulate in the faeces of preschool children and their parents and which are the associated risk factors. Furthermore, we aimed to assess the associations between enteropathogen carriage and clinical symptoms, as well as the associations of the same enteropathogens between children and their parents to unveil possible household transmission.

Methods

Study design

From April 2013 to October 2014, we performed a monthly-repeated cross-sectional survey of potential AGE-causing agents in households with children of preschool age (≤4 years) in the Netherlands. Each month for a period of 19 months, 2000 preschool children were selected at random (without replacement) from continuously-updated population registries of 335/415 municipalities covering 78% of the Dutch population (~16.8 million, ~5% aged ≤4 years); only one child per household could be sampled. The parents of the selected children were invited to complete an online epidemiological questionnaire and to provide a stool sample from the selected child and from one freely-selected parent living in the household. The questionnaire contained questions about demographic and household characteristics, DCC attendance, history of chronic diseases and medications, contact with animals, leisure activities, and eating habits. Questions referred to the 30 days prior to questionnaire completion. More information on the survey can be found in a previous open-access publication (6). Questions covered the previous four weeks (recall period). The degree of urbanization and socio-economic status (SES) were obtained at the postcode level from Statistics Netherlands (http://www.cbs.nl/en-GB/menu/home/default.htm).

Faecal sample collection

Upon completion of the questionnaire, the participants were invited to collect and submit a stool sample regardless of the presence of clinical symptoms related to AGE. If the participant was willing to do so, we provided a stool sample collection kit consisting of two pre-labelled sterile tubes (one for the child and one for the parent) together with informative material on how to collect and send back the samples to the laboratory using a pre-stamped envelope. An additional short questionnaire was also provided in order to check whether any new symptom or treatment had occurred since the completion of the first questionnaire; this additional questionnaire referred to the two weeks prior to stool sampling.

Case definition

We used an AGE case definition based on the symptoms reported in the second questionnaire referring to the two weeks prior to stool sampling (7). An AGE case was defined as a person with ≥3 diarrhoeal discharges and/or any clinically relevant

vomiting (in absence of pregnancy) in 24 h during the previous two weeks, excluding those with chronic enteropathies (e.g. bowel cancer, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis, coeliac disease, Crohn's disease, food allergy/intolerance, malabsorption syndromes, gastroesophageal reflux disease, chronic gastritis, and peptic ulcer disease).

Sample processing and DNA extraction

Faecal suspensions were prepared as described earlier (8). DNA and RNA were extracted from the faecal suspension using the automated NucliSENS easyMAG (bioMérieux) according to the manufacturer's instructions. In addition, phocine herpesvirus 1 (PhHV) and equine arteritis virus (EAV), which served as an internal control, were co-purified.

Detection of bacteria and parasites

All tested targets are listed in Table 1. The bacterial and parasitic enteropathogens tested for were *Salmonella* spp., *Yersinia enterocolitica*, *Shigella* spp./ enteroinvasive *Escherichia coli* (EIEC), *Campylobacter jejuni* and *C. coli*, *Clostridium difficile* toxin, *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica*. A number of bacterial pathogenicity genes were also tested for (Table 1). The ability to produce Shiga toxins (Stx) is a common characteristic of all Stx-producing *E. coli* (STEC); the genes encoding Stxs are *stx1* and *stx2*. STEC contains a variety of candidate virulence factors. These include, in addition to Stx, several pathogenicity islands such as the locus of enterocyte effacement (LEE) containing genes responsible for the characteristic attaching and effacing (A/E) lesions, e.g. *escV*. The *escV* gene is a marker for atypical enteropathogenic *E. coli* (aEPEC). The *bfpA* gene is a marker gene for enteropathogenic *E. coli* (EPEC); the *aggR* gene is a specific marker for enteroaggregative *E. coli* (EAEC).

Real-time multiple polymerase chain reaction (mPCR) was carried out on an AB7500 sequence detection system (Applied Biosystems) in five multiplex target sets. For every multiplex set, the Ct value for the PhHV was monitored and an mPCR was considered inhibited when the Ct value exceeded the mean Ct value +2 standard deviations. Inhibition of PCR due to impurities in the faecal extracts was at most 3.7% and these samples were considered 'not tested' in the data analysis (unless the target was tested positive), resulting in a different number of total samples analysed for each target.

Detection of viruses

The detection of norovirus genogroups I and II, sapovirus, astrovirus, rotavirus, and adenovirus type 41 was performed as described earlier (9), with some minor modifications. For reverse transcription polymerase chain reaction (RT-PCR), a one-step procedure was performed with TaqMan Fast Virus 1-Step Master Mix. The primers and probes we used are listed in Table S1. In addition to the generic adenovirus PCR, a specific PCR for adenovirus type 41 was implemented and only this one was analysed for this study since the other types are not strictly associated with AGE. PCR amplification was performed on a LightCycler 480 instrument (Roche Diagnostics GmbH). After the RT reaction for 1 h at 50°C, samples were denatured for 1 min at 95°C and subjected to 50 cycles at 95°C for 10 s and 60°C for 30 s for amplification. Seventeen samples (less than 1%) showed moderate levels of inhibition for both the EAV and the PhHV internal controls; in eight of them, viral nucleic acid was, nevertheless, detected.

Data analysis

As mentioned before, technically failed/inhibited samples were excluded from further analyses of each set of targeted agents. However, in cases of one failed sample in a child-parent pair, the other (non-failed) sample was used in the prevalence and risk factor analyses, as these were performed independently for parents and children (see below), but the non-failed sample was then automatically excluded when assessing the co-carriage (between children and their parents) of the same enteropathogens. All bacterial and parasitic PCR results with a Ct value <40 and exponential S curve signal were considered positive. For the viral targets, a Ct value <50 was considered positive.

Risk factor analysis was performed as described previously (6). Potential risk factors for the presence of each agent were first assessed using single-variable logistic regression models. Factors showing a p-value ≤0.10 for the association with the outcome were included in a multivariable logistic regression model; the variables age (0–12, 13–36, ≥37 months), gender, urbanisation degree (>2500, 500–2500, <500 addresses/km^2) and season (spring, March–May; summer, June–August; autumn, September–November; winter, December–February) were always included in the model to control for potential confounding effects. A backward stepwise variable selection procedure was then applied to retain variables with a p-value <0.05, provided that the removal of these variables from the model did not change significantly the associations of the other covariates. Collinearities were identified

prior to multivariable analysis and selection between collinear variables was made based on the Akaike's information criterion (AIC). A complete record analysis was performed. Associations were expressed as odds ratios (ORs) providing 95% confidence intervals (95%CIs). Since fewer associations were found for parents, the results were not presented in table format, but all significant factors are described in the text of the results section. For models with limited sample sizes (<10 outcome events per variable), bias-corrected bootstrap 95%CIs (1000 replications) were also calculated and compared with the standard ones to support the inference and generalisability of the fitted models (10). Logistic regression analysis was also used to assess the associations between having a child positive for a given agent and detecting the same agent in the respective parent's stool, as well as the associations between each pathogen carriage and different gastrointestinal symptoms (vomiting, diarrhoea, abdominal cramps, blood in the stool, mucus in the stool, discoloration in the stool), including AGE as a syndrome.

Results

In total, 49,732 child-parent pairs were invited to participate in the study and 10,109 (20.3%) completed the questionnaire. In total, 946 (9.4%) pairs were discarded because the child was older than 48 months (the age at which children are eligible to enrol in primary school in the Netherlands), because the parents had reported data for a child other than the one invited to participate, or because the questionnaire was filled in inconsistently (unreliable/conflicting answers denoting mixing up of answers for children and parents) as reported previously (6). Another 395 (3.9%) pairs were discarded because the questionnaire was incomplete (missing entries), leaving 8768 pairs enrolled in the study. Among the respondents, 33.4% were willing to submit the faecal samples; 1952 stool samples (981 from children and 971 from parents) were eventually received and analysed for the bacterial and parasitic targets, and 1843 of them (928 from children and 915 from parents) were tested also for viral targets. The median age of the children (49% males) was 28 months (interquartile range [IQR] 17–39 months), and 34 years (IQR 31–37) for the parents (16.1% males). Inhibition of PCR due to impurities in the faecal extracts was negligible.

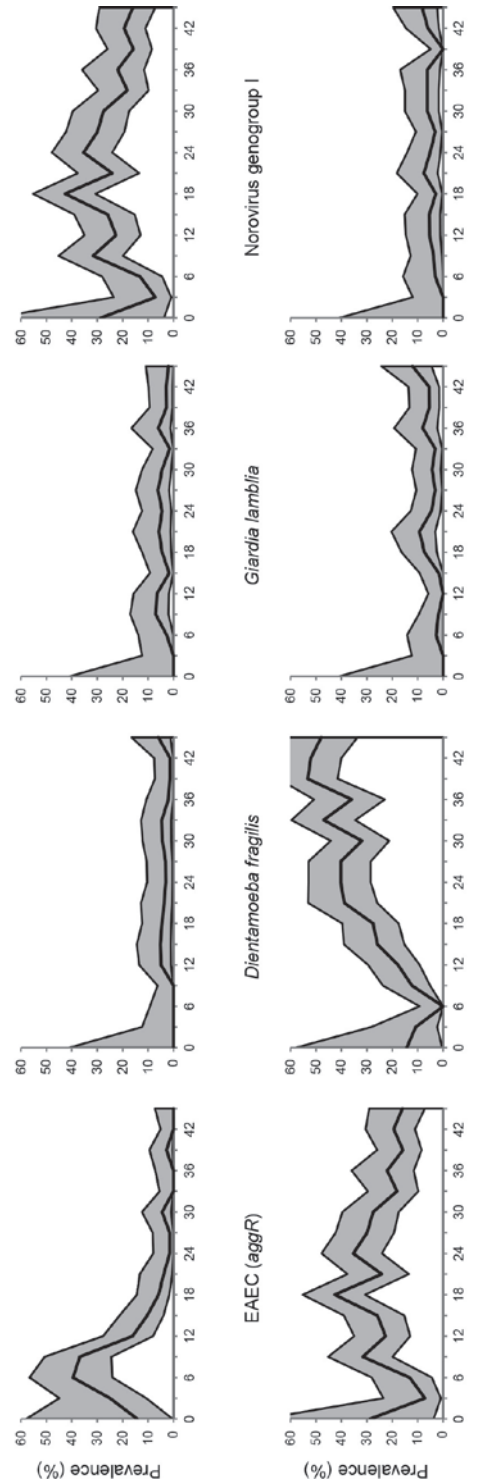

Figure 1. Percentage of stool samples tested positive for bacterial, parasitic and viral enteropathogens per age group of 3 months, with corresponding 95% confidence intervals.

Table 1. Prevalence of the enteropathogens tested for and their associations between children and their parents

	***n* tested**	***n* positive**	**Children** *n* (%)	**Parents** *n* (%)	**OR[a] (95%CI)**
Bacteria					
Salmonella spp.	1879	1	0 (0)	1 (0.1)	nc
Yersinia enterocolitica	1901	4	2(0.2)	2(0.2)	nc
Shigella spp./EIEC	1886	0	0(0)	0(0)	nc
Campylobacter jejuni	1879	7	3(0.3)	4(0.4)	nc
Campylobacter coli	1886	0	0(0)	0(0)	nc
Clostridium difficile toxin	1887	87	74(7.7)	13(1.4)	ns
Bacterial pathogenicity genes					
Shiga-like toxin 1/2 (STEC)	1891	48	27(2.8)	21(2.3)	8.1(2.4-28)
escV (a-EPEC)[b]	1900	341	234(24.2)	107(11.5)	2.5(1.6-3.8)
bfpA (t-EPEC)	1886	43	37(3.9)	6(0.7)	14.9(2.6-84.4)
aggR (EAEC)	1900	78	47(4.9)	31(3.3)	40.2(17.6-91.6)
Viruses					
Norovirus genogroup I	1843	70	40(4.3)	30(3.3)	18.5(7.9-43.5)
Norovirus genogroup II	1843	84	60(6.5)	24(2.6)	17.9(7.2-44.9)
Sapovirus	1843	54	37(4)	17(1.9)	98.6(28.2-344.2)
Astrovirus	1843	23	16(1.7)	7(0.8)	98.7(19.9-489)
Rotavirus	1843	22	12(1.3)	10(1.1)	ns
Adenovirus type 41	1843	8	7(0.1)	1(0.8)	nc
Parasites					
Dientamoeba fragilis	1922	721	349(36)	374(39.2)	2.2(1.6-2.9)
Giardia lamblia	1890	67	44(4.6)	23(2.5)	34.8(13.9-86.7)
Cryptosporidium spp.	1922	2	1(0.1)	1(0.1)	nc
Entamoeba histolytica	1922	0	0(0)	0(0)	nc

ns = P> 0.05 not significant, nc = not calculable, too few cases. OR = odds ratio, 95%CI = 95% confidence interval;

[a]Derived using multivariable logistic regression models, including all significant risk factors as reported in the text as covariates; [b]22/27 children and 8/21 parents were tested positive for both Shiga-like toxin and *escV*-genes.

Bacteria

Campylobacter jejuni/coli, Y. enterocolitica and *Salmonella* spp. were only sporadically detected (<0.4%) in both parents and children (Table 1). The gene for *C. difficile* toxin was detected in 7.7% of children, but mostly in children between 6 and 18 months of age (Table 1 and Fig. 1). For none of these pathogens was an association within the household found.

Of the tested *E. coli* virulence genes, a-EPEC was the most prevalent, being detected in 24.2% of the children and 11.5% of the parents, and was associated with

summertime in children and parents (Table 1, parents' OR 2.2, 95% CI 1.3-3.9), whereas t-EPEC was detected in only 3.9% of children and 0.7% of parents (Table 1). In male parents, t-EPEC was found more often compared to females (OR 2.3, 95% CI 1.1–4.6). EAEC was detected in 4.9% of children and 3.3% of parents, while STEC was detected in 2.8% of children and 2.3% of parents (Table 1). Furthermore, having siblings was protective for *C. difficile* detection in children (OR 0.49, Table 2).

Detection of STEC was associated with having farm animals (OR 2.9). All the aforementioned *E. coli* virulence genes but EAEC were more often detected in summer than in winter (Table 2). Living in rural areas (OR 4.1) and having horses around the house (OR 9.7) or around the DCC (OR 11.8) were associated with positivity to EAEC (Table 2). All *E. coli* virulence genes were found significantly more often in parents of positive children (OR 2.5 to 40.2, Table 1). In 1.4% of the parents, *C. difficile* toxin genes were detected, but none had a positive child.

Parasites

Dientamoeba fragilis was the most common parasite, being detected in 36% of children and 39% of parents, followed by *G. lamblia* (4.6% of children and 2.5% of parents, Table 1). *Cryptosporidium* spp. was rarely (0.1%), and *E. histolytica* was never, detected (Table 1). *Dientamoeba fragilis* was associated with increasing age and number of siblings in the household in both children (Table 3 and Fig. 1) and parents (two children: OR 1.7, 95% CI 1.2–2.4, three children: OR 3.5, 95% CI 2.3–5.2). Also living in lowly urbanised areas (OR 1.8) and having a dog (OR 1.6) were associated with *D. fragilis* detection in children (Table 3). Attending a DCC (OR 2.6) and having a sandpit (OR 4.2) were associated with being positive for *G. lamblia* in children. Moreover, the risk of *G. lamblia* detection was even higher (OR 5.1) in children attending DCCs with animals. Having a cat in the household was associated with *G. lamblia* detection in both children (OR 2.1) and parents (OR 2.5, 95% CI 1.1–5.7) (Table 3). Having a *D. fragilis*- or *G. lamblia*-positive child was associated with increased odds (OR 2.2 and 34.8) of detecting the same parasite in parents (Table 1).

Viruses

Caliciviridae (norovirus genogroups I and II, and sapovirus) were often detected in children (4.3%, 6.5%, and 4%, respectively) and parents (3.3%, 2.6%, and 1.9%, respectively) (Table 1, Fig. 1d). Astrovirus, rotavirus, and adenovirus were found in 1.7%, 1.3%, and 0.1% of children, and 0.8%, 1.1% and 0.8% of parents, respectively (Table 1). Caliciviridae were associated with attending DCCs (Table 4). All viruses

except norovirus genogroup I were significantly more often detected in winter and spring than in summer in children (Table 4). In parents, only norovirus genogroup II was associated with autumn (OR 7.1 95% CI 1.4–37) and winter (OR 14.1 95% CI 3.2–62.7), and sapovirus with winter (OR 13.6 95% CI 1.7–110.9) and spring (OR 18.6 95% CI 2.3–152.6) compared to summer. Norovirus genogroup I was less likely to be detected in male than female children (OR 0.4), but it was more likely to be detected in children living in rural areas (OR 3.1) and in those owning animals (OR 2.5) (Table 2b). For parents, the detection of norovirus genogroup I was associated with living in rural areas (OR 8.8, 95% CI 1.8–43.7) and owning poultry or birds (OR 2.9, 95% CI 1.1–8.2).

Detection of noroviruses, sapovirus, and astrovirus in children was strongly associated with detecting the same viruses in parents (Table 1).

Clinical symptoms

In the two weeks prior to sampling, 22.7% of children experienced AGE (18.3% diarrhoea and 8.3% vomiting). Of all pathogens tested for, norovirus genogroup II, astrovirus and adenovirus type 41 were detected significantly more often in children with AGE and were thus associated with clinical symptoms (Table 5). For *C. jejuni*, t-EPEC and *Cryptosporidium* spp., the data pointed to an association with AGE in children. In total, 16.3% of parents experienced AGE. Only norovirus genogroup II, sapovirus and *Cryptosporidium* spp. was significantly associated with AGE (Table 5).

Table 2. Multivariable odds ratios and corresponding 95% confidence intervals of the significant risk factors for detection of bacterial enteropathogens in children

	Cases (%)	***C. difficile* toxin**	**STEC**	**a-EPEC**	**t-EPEC**	**EAEC**
Season						
Summer	41.1	ns	11.7 (1.6-87.6)	2.7 (1.8-4.1)	2.8 (0.9-8.4)	ns
Autumn	19.3	ns	nc	2.6 (1.6-4.2)	4.7 (1.5-14.7)	ns
Winter	23.6	ns	Reference	Reference	Reference	ns
Spring	16.0	ns	nc	0.4 (0.2-0.8)	nc	ns
Child age[a]						
0-12	33.1	54.6 (16.5-180.5)	ns	nc	ns	ns
13-36	50.6	5.9 (1.8-19.5)	ns	1.7(1.2-2.5)	ns	ns
≥37	16.3	Reference	ns	Reference	ns	ns
Gender♂/♀	49.7/50.3	ns	ns	ns	2.3 (1.1-4.6)	ns
Urbanisation						
High	24.4	ns	ns	ns	ns	Reference
Moderate	63.1	ns	ns	ns	ns	nc
Rural	12.6	ns	ns	ns	ns	4.1 (1.5-10.8)
N children[b]						
1	29.5	Reference	ns	ns	ns	Reference
2	49.6	0.5 (0.3-0.9)	ns	ns	ns	2.4 (1.1-5.4)
3or more	20.6	0.5 (0.2-1.1)	ns	ns	ns	nc
Animals						
Livestock[c]	11.0	ns	2.9 (1.2-7.3)	ns	ns	ns
Equids[d]	2.8	ns	ns	ns	ns	9.7 (3.2-26.9)
Day-carecentre	52.1	ns	ns	ns	ns	ns
With animals	19.1	ns	ns	ns	ns	ns
With equids[d]	3.0	ns	ns	ns	ns	11.8 (2.9-49)

ns = P> 0.05 not significant, nc = not calculable, too few cases

[a]Age of children in months; [b]Number of children within the household; [c]Farms animals included poultry, horses, cattle, pigs and rabbits; combined analyses since most households had more types of animals; [d]Includes horses, ponys and/or donkeys

Table 3. Multivariable odds ratios and corresponding 95% confidence intervals of the significant risk factors for detection of parasites in children

	Cases (%)	***Giardia lamblia***	***Dientamoeba fragilis***
Child age[a]			
0-12	33.1	ns	Reference
13-36	50.6	ns	5.6 (2.8-11.2)
≥37	16.3	ns	10.2 (5-20.7)
Urbanisation			
High	24.4	ns	Reference
Moderate	63.1	ns	nc
Rural	12.6	ns	1.8 (1-3)
N children[b]			
1	29.5	ns	Reference
2	49.6	ns	1.8 (1.2-2.6)
3ormore	20.6	ns	6.4 (4.1-10)
Animals			
Dogs	16.1	ns	1.6 (1.1-2.4)
Cats	27.6	2.1 (1.1-3.9)	ns
Day-care centre	52.1	2.6 (1.2-5.5)	ns
With animals[c]	19.1	5.1 (2-12.6)	ns
Sandpit at home	58.8	4.2 (1.8-10.2)	ns

ns = P> 0.05 not significant, nc = not calculable, too few cases

[a]Age of children in months; [b]Number of children within the household; [c]Having any animal (outdoor or indoor)

Table 4. Multivariable odds ratios and corresponding 95% confidence intervals of the significant risk factors for detection of viral enteropathogens in children

	Cases (%)	**Norovirus I**	**Norovirus II**	**Sapovirus**	**Astrovirus**	**Rotavirus**
Season						
Summer	41.1	ns	Reference	Reference	Reference	Reference
Autumn	19.3	ns	3.3 (1.5-7)	nc	nc	nc
Winter	23.6	ns	3.5 (1.7-7.2)	3.3 (1.2-9.3)	20.2 (2.5-162)	nc
Spring	16.0	ns	nc	7.2 (2.7-18.9)	13 (1.5-113.4)	9.4 (1.9-45.7)
Child age[a]						
0-12	33.1	ns	3.1 (1.4-6.5)	ns	ns	ns
13-36	50.6	ns	nc	ns	ns	ns
≥37	16.3	ns	Reference	ns	ns	ns
Gender♂/♀	49.7/50.3	0.4 (0.2-0.8)	ns	ns	ns	ns
Urbanisation						
High	24.4	Reference	ns	ns	ns	ns
Moderate	63.1	nc	ns	ns	ns	ns
Rural	12.6	3.1 (1.1-9)	ns	ns	ns	ns
Animals						
Livestock[b]	11.0	2.5 (1-6)	ns	ns	ns	ns
Day-care centre	52.1	2.3 (1.1-4.9)	1.9 (1.1-3.4)	2.2 (1.1-4.7)	ns	ns

ns = P> 0.05 not significant, nc = not calculable, too few cases

[a]Age of children in months; [b]Farms animals included poultry, horses, cattle, pigs and rabbits; combined analyses since most households had more types of animals

Discussion

This study shows a substantial overlap of pathogens, particularly viruses, in the stools of preschool children and their parents, suggesting that household transmission of these agents occurs extensively. While bacteria are likely to play a major role during the summer, viruses predominate in the winter, but parasites do not seem to exhibit a significant seasonal pattern. The design of this study does not allow for conclusions about directionality of transmission between children and parents to be drawn, as the detection of the same pathogen in both children and parents might also reflect a common source of infection. However, previous research has shown that secondary transmission of AGE-causing agents occurs often from children to their parents (5). Moreover, the association between DCC attendance and increased odds for virus and *G. lamblia* detection in the stool is suggestive of entry of these pathogens into the household via the DCC-attending children (11-14).

Several other caveats also need to be considered. The prevalence estimates reported here might be overestimated because we only looked at genetic material of the pathogens in the stool and it is known that this can be detected in the faeces of cured patients for several weeks (3). Moreover, the timing for the parents to collect the stool samples after filling in the first questionnaire might have been biassed by the onset of symptoms, as more AGE cases (22.7% in children and 16.3% in parents) seemed to occur in this 2-week period compared to the period of the first (17.3% in children and 7.3% in parents), though there might have been a better recall for symptoms for a period of 2 weeks in contrast to 4 weeks.

As mentioned in the introduction, a questionnaire-based study investigating the societal burden (i.e. incidence, healthcare utilisation, and productivity loss) and correlates of self-reported AGE (as a syndrome) in households with preschoolers has been published using the same cohort used in the present study (6). This former study reported an incidence rate of 0.95 episodes of AGE per parent/year and 2.25 episodes of AGE per child/year, with 18.3% of children and 8.6% of parents requiring medical attention, leading to an overall AGE-related work absenteeism of 55.6%. Significant correlates of childhood AGE were chronic gastrointestinal diseases, gastric antacid use, non-breastfeeding, toddling age, developmental disabilities, parental occupation in healthcare, multiple siblings, single-parent families, and ≤12-month DCC attendance, while parental AGE was associated with female gender, multiple or developmentally-disabled DCC-attending children, antimicrobial use, and poor food-handling practices.

Table 5. Enteropathogen detections in the stool samples of subjects with and without AGE symptoms in the 2 weeks prior to sampling

Pathogen	**AGE +** *N*+/*n* tested (%)	**AGE -** *N*+/*n* tested (%)	***P****
Children			
Salmonella spp.	0/214 (0)	0/744 (0)	
Yersinia enterocolitica	0/217 (0)	2/751 (0.3)	0.5
Shigella spp./EIEC	0/217 (0)	0/746 (0)	
Campylobacter jejuni	2/214 (0.9)	1/744 (0.1)	0.07
Campylobacter coli	0/216 (0)	0/744 (0)	
C. difficile toxin	17/216 (7.9)	57/744 (7.7)	0.9
Shiga-like toxin 1/2 (STEC)	5/217 (2.3)	22/746 (3)	0.6
escV (a-EPEC)	50/217 (23)	184/751 (24.5)	0.6
bfpA (t-EPEC)	13/216 (6)	24/744 (3.2)	0.06
aggR (EAEC)	12/217 (5.5)	35/ 751 (4.66)	0.6
Norovirus genogroup I	9/212 (4.3)	31/716 (4.3)	1
Norovirus genogroup II	26/212 (12.3)	34/716 (4.8)	**<0.01**
Sapovirus	8/212 (3.8)	29/716 (4.1)	0.9
Astrovirus	8/212 (3.8)	8/716 (1.1)	**<0.01**
Rotavirus	4/212 (1.9)	8/716 (1.1)	0.4
Adenovirus type 41	5/212 (2.4)	2/716 (0.3)	**<0.01**
Dientamoeba fragilis	71/218 (32.6)	278/751 (37)	0.2
Giardia lamblia	6/215 (2.8)	38/744 (5.1)	0.2
Cryptosporidium spp.	1/218 (0.5)	0/751 (0)	0.06
Entamoeba histolytica	0/218 (0)	0/751 (0)	
Parents			
Salmonella spp.	0/147 (0)	1/774 (0.1)	0.7
Yersinia enterocolitica	1/149 (0.7)	1/783 (0.1)	0.2
Shigella spp./EIEC	0/148 (0)	0/780 (0)	
Campylobacter jejuni	2/147 (1.4)	2/774 (0.3)	0.06
Campylobacter coli	0/216 (0)	0/744 (0)	
C. difficile toxin	4/148(2.7)	9/779 (1.2)	0.1
Shiga-like toxin 1/2 (STEC)	5/148 (3.4)	16/780 (2.1)	0.3
escV (a-EPEC)	19/149 (12.8)	88/783 (11.2)	0.6
bfpA (t-EPEC)	1/147 (0.7)	5/779 (5)	1
aggR (EAEC)	7/149 (4.7)	24/783 (3.1)	0.3
Norovirus genogroup I	7/145 (4.8)	23/770 (3)	0.3
Norovirus genogroup II	10/145 (6.9)	14/770 (1.8)	**<0.01**
Sapovirus	6/145 (4.1)	11/770 (1.4)	**0.03**
Astrovirus	1/145 (0.7)	6/770 (0.8)	0.9
Rotavirus	0/145 (0)	10/770 (1.3)	0.2
Adenovirus type 41	0/145 (0)	1/770 (0.1)	0.7
Dientamoeba fragilis	51/153 (33.3)	323/800 (40.4)	0.1
Giardia lamblia	6/147 (4.1)	17/774 (2.2)	0.2
Cryptosporidium spp.	1/153 (0.7)	0/800 (0)	**0.02**
Entamoeba histolytica	0/153 (0)	0/800 (0)	

*Pearson chi^2

This study, however, did not look at the specific enteropathogens circulating in these households, nor did it relate the carriage of these enteropathogens to potential risk factors, their clinical relevance and evidence for household transmission, all of which were addressed in the present study.

Owning farm animals was a risk factor for detecting STEC in children, and summertime was a risk factor for both STEC and EPEC. It is known that ruminants and, specifically, cattle are the main reservoir for STEC (15). In summer, outdoor keeping of dairy cattle is common and beef calving season is ongoing. This results in increased environmental contamination with cattle faeces (16), which coincides with increased STEC detection in humans. Surprisingly, exposure to horses was associated with increased odds of detecting EAEC in children's stools. This association was not significant in parents, though they were likely to acquire EAEC from their children. To our best knowledge, humans have so far been the only recognised reservoir for EAEC, calling for further investigations on the role of horses.

Toxin-producing *C. difficile* is an important cause of healthcare-associated diarrhoea. In accordance with previous reports, we found a higher *C. difficile* prevalence in children under 2 years of age, with the highest prevalence at the age of 9 months (17). Since our molecular target for *C. difficile* was based on genes for toxins A/B, our results only represent potentially toxicogenic strains. Studies in other European countries showed that toddlers can harbour potentially pathogenic ribotypes of *C. difficile* (18, 19). As multiple (healthy) young children have detectable toxicogenic *C. difficile* in their stools, there is indication of high contamination pressure from their immediate environments. These children might also act as sources of infection for the immunocompromised and elderly. Yet, our data do not show any increased odds for *C. difficile* detection in the parents, a possible indication of low susceptibility to *C. difficile* in our (young) adult population.

DCC attendance has long been identified as a risk factor for gastrointestinal infections (20). Our study shows that children attending DCC are more likely to have norovirus, sapovirus and astrovirus in their stools, and that these viruses are associated with AGE symptoms, emphasising the clinical relevance of these viral detections. Although half of the children in our study did not attend DCCs, we found a slightly higher prevalence for viruses as compared to that of a previous Dutch study focussed on DCC-attending children (4). This can possibly be explained by selection bias due to preferential sampling at the time of symptoms as mentioned above.

This study also confirms previous findings that attending a DCC and having access to sandpits are risk factors for *G. lamblia* detection in children's stools (21). This is the first time, however, that owning cats was identified as a risk factor for *G. lamblia*, although it is known that cats can be colonised with human-associated *G. lamblia* assemblages and that cats have a propensity for using sandpits as defecation area (22). For most tested pathogens, we did not find an association with AGE symptoms, confirming the notion that the gut of young children often gets colonised by potentially harmful microorganisms without eliciting a symptomatic response. This calls into question the clinical significance of molecular detection of these pathogens. For *D. fragilis,* the rate of colonisation was higher in children and parents without AGE, supporting the ongoing debate about its pathogenicity (23).

In conclusion, we show that different enteropathogens circulate in households with preschool children in a Western, high-income country like the Netherlands. Viral detections seem to have the highest clinical relevance in regard to AGE symptoms. Previously documented risk factors like age, attending DCCs, use of sandpits, and season were confirmed but, also, novel factors were reported (e.g. proximity to horses for EAEC, ownership of cats for *G. lamblia*, etc.), providing insights for new research questions and potential targets for AGE-reducing initiatives.

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CHAPTER 5

Clinical relevance of enteropathogen co-infections in preschool children – a population-based repeated cross-sectional study

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Published in Clinical Microbiology and Infection, 2018

Abstract

Background

This study aimed to (i) determine risk factors for enteropathogen co-infections, (ii) determine whether enteropathogen co-infections influence gastroenteritis risk, and (iii) determine whether enteropathogen co-infection occurred randomly in preschool children.

Methods

A monthly-repeated cross-sectional survey in Dutch children aged 0-48 months was conducted during October 2012-October 2014. A total of 981 stool samples were collected along with questionnaires collecting data on gastrointestinal symptoms and potential risk factors. 822 samples were successfully tested for 19 enteropathogens using real-time multiplex PCRs. Logistic regression analysis assessed co-infections in relation to gastroenteritis and potential risk factors.

Results

In all, 598/822 (72.7%) stool samples tested positive for at least one enteropathogen, of which 290 (48.5%) were positive for two or more enteropathogens. Risk factors for two or more enteropathogen co-infections were young age (<12 months, OR 1.9, 95%CI 1.1-3.3; 13-36 months, OR 1.7, 95%CI 1.1-2.5, *vs*. 37-48 months), day-care attendance (OR 1.8, 95%CI 1.3-2.5), households with ≥3 children versus those with one child (OR1.7, 95%CI 1.1-2.8). Stool samples collected in spring less often had two or more enteropathogens versus summer (OR 0.4, 95%CI 0.2-0.7). Food allergy was a risk factor for three or more enteropathogen co-infections (OR 3.2, 95%CI 1.1-8.9). The frequency of co-infection was higher than expected for norovirus GI/norovirus GII, *Clostridium difficile*/norovirus GI, *C. difficile*/rotavirus, astrovirus/*Dientamoeba fragilis*, atypical enteropathogenic *Escherichia coli*/adenovirus, typical enteropathogenic *E. coli*/adenovirus, and enteroaggregative *E. coli*/astrovirus. No co-infection was associated with increased gastroenteritis risk.

Conclusions

Risk factors for enteropathogen co-infections were identified and specific enteropathogens co-occurred significantly more often than expected by chance. Enteropathogen co-infections were not associated with increased gastroenteritis risk, calling into question their clinical relevance in preschool children.

Introduction

Gastroenteritis (GE) is one of the most common diseases in developing countries and causes significant morbidity in developed countries as well, particularly among preschool children (1). Among children hospitalized for GE in the Netherlands, co-infections of viruses, bacteria and parasites were detected in 40% of cases (2). Other European countries report widely varying rates of co-infections in children hospitalized for GE (3-7). Available data are limited and inhomogeneous, however, testing for different panels of enteropathogens. Furthermore, it is unclear whether children with co-infections have increased risk for (severe) GE, as the literature shows conflicting results.

A study among children admitted to hospital for GE in Italy and children with GE in hospital emergency rooms in Spain found that those with co-infections had more severe clinical pictures (3, 6). This is in contrast with other studies among hospitalized children in Italy, France and Brazil (7-9). In the Netherlands, mixed infections were not associated with specific symptoms in children hospitalized for GE (2). Because these studies focus on children hospitalized for GE, they can only associate co-infections with disease severity, but cannot assess whether children with co-infections are more likely to have GE *per se*. Although this requires studies on co-infections in children with and without GE, these are scarce in the literature. Furthermore, if enteropathogens could occur independently of each other, they would occur at random in all children. However, possible interactions between enteropathogens have been described but are poorly understood (10).

Focusing on preschool children in the general population, the objectives of this study were to (i) identify risk factors for enteropathogen co-infections in children in the general population, (ii) determine whether enteropathogen co-infections influence GE risk, and (iii) determine whether specific combinations of co-infecting enteropathogens occur significantly more or less often than expected.

Methods

Study design and population

We used data from a monthly-repeated cross-sectional survey in the Netherlands conducted during October 2012 to October 2014. For a detailed description of its design, we refer to previously published papers (1, 11). In short, 2000 families

with preschool children <4 years were randomly selected from Dutch municipal population registries every month for a period of 25 months. They were invited to complete a web-based questionnaire regarding household characteristics and health status in the past 4 weeks for the sampled child and one freely chosen parent in the household. If they were willing to submit a stool sample, we provided a sample collection kit with an additional questionnaire to check whether new symptoms had occurred in the 2 weeks before to stool sampling.

Gastroenteritis case definition

A GE case was defined as a child with ≥3 diarrheal discharges in 24 hours or any " clinically relevant" vomiting during the previous two weeks prior to stool sampling, according to a standard GE case definition (12). By "clinically relevant" vomiting we refer to vomiting events other than regurgitation, vomiting due to motion sickness/ vertigo, intense exercise, traumatic or nauseous events.

Detection of bacteria, parasites and viruses

Stool samples were tested for several viruses, bacteria and parasites using internally controlled quantitative real-time multiplex polymerase chain reactions (qPCRs) as described previously (13-16). Viruses tested for were norovirus genogroups I (GI) and II (GII), sapovirus, astrovirus, rotavirus, adenovirus, and adenovirus type 41, and bacteria were *Salmonella* spp., *Yersinia enterocolitica*, *Shigella* spp./ enteroinvasive Escherichia coli (EIEC), *Campylobacter jejuni/coli* and *Clostridium difficile* toxin A/B. Bacterial pathogenicity genes tested for were Shiga toxins (*stx)* 1 and *stx*2, *bfpA*, *escV*, and *aggR* . *Stx* is a common characteristic of Shiga toxins-producing *Escherichia coli* (STEC). *BfpA* is a marker gene for enteropathogenic *E.* coli (EPEC), *escV* for atypical enteropathogenic *E. coli* (a-EPEC) and *aggR* for enteroaggregative *E. coli* (EAEC). Parasites tested for were *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptospordium* spp. and *Entamoeba histolytica*.

Data analysis

For each enteropathogen, we determined the observed and expected frequencies of co-infection with any of the other enteropathogens in the same children. The expected frequency was calculated as the weighted sum of the products of the age-specific (0-12, 13-36 and 37-48 months) prevalence rates of these two enteropathogens. We tested for differences between the observed and expected co-infection prevalence rates using two-sample Z-tests for proportions. To minimize spurious associations

between enteropathogens due to shared risk factors, those with a p-value <0.10 were entered in a multivariable logistic regression model (one enteropathogen as binary dependent variable and the other as binary independent variable) to be adjusted for shared risk factors. These factors were selected based on previous risk analyses on the same dataset (11). Correlations between enteropathogens were visualized using principal component analysis (PCA; see Supplementary Figure S1).

We examined for each enteropathogen whether co-infection with each of the other enteropathogens was associated with GE. A separate multivariable logistic regression model was built for each pair of enteropathogens, including GE as the dependent variable and the two enteropathogens as interaction term. Children with no enteropathogens detected were the reference group. If the combination of two enteropathogens was significantly associated with GE (p-value <0.05), we also compared the combination with single infections of both enteropathogens. All analyses were adjusted for age and underlying enteropathies. Because *C. difficile* infections are more often asymptomatic in children <2 years, we performed age-stratified analyses for *C. diffile* co-infections (17).

Finally, we built univariate logistic regression models to examine associations between demographics and household characteristics (listed in Table 1) with co-infection of ≥2 enteropathogens compared with only one enteropathogen. We did the same for co-infection of ≥3 enteropathogens. Variables with a p-value of <0.10 were entered in a multivariable model built using backward stepwise selection. In multivariable analyses, a p-value of <0.05 was considered to be statistically significant. Analyses were performed using STATA version 14.2 (College Station, TX, USA).

Table 1. Demographics and household characteristics of the study population (n=822)

	N	%
Demographics		
Age		
<12 months	120	14.6
13-36m	470	57.2
37-48m	232	28.2
Female	408	49.7
Non-Western migration background[a]	37	4.5
Enteropathies	68	8.3
Food allergy	36	4.4
Reflux	34	4.1
Bowel disorder	6	0.7
Day-care attendance	434	52.9
Use of gastric acid inhibitors[b]	6	0.7
Use of antibiotics[b]	66	8.0
Hospitalization[c]	0	0
Having gastroenteritis	185	22.5
Season[d]		
Spring	145	17.6
Summer	353	42.9
Autumn	144	17.5
Winter	180	21.9
Household characteristics		
Number of children in household		
1	251	30.6
2	397	48.4
≥3	173	21.1
Breastfeeding		
None	181	22.1
<3 months	179	21.8
3-6 months	234	28.5
>6 months	223	27.2
Unknown	4	0.5
Urbanization degree		
<500 addresses/km^2	108	13.2
500-2500 addresses/km^2	502	61.3
>2500 addresses/km^2	209	25.5
Having a pet dog	137	16.7
Having a pet cat	222	27.0
Having farm animals	89	10.8
Having a sandpit	478	58.2

[a]The child or one of its parents is not born in Europe (excluding Turkey), North America, Oceania, Indonesia or Japan; [b]In the past six months; [c]In the past four months due to GE; [d]Spring: March to May, summer: June to August, autumn: September to November, winter: December to February

Results

In total, 49,732 households were invited and 10,109 (20.3%) filled in the questionnaire. Of these, 946 (9.5%) were excluded because the child was older than 48 months, the parent had reported data for a child other than the one invited, or the questionnaire was inconsistently filled in. Faecal samples were obtained from 981 children (9.7%) and enteropathogen detection (positive or negative) was successfully performed for the entire panel of enteropathogens in 822 samples (83.3%). The median age of children was 28 months (interquartile range: 17-38 months) and 408 (49.7%) were female (Table 1). A total of 185 children (22.5%) experienced GE within 2 weeks of to stool sampling.

Co-infection

In 224 children (27.3%), no enteropathogens were detected, in 308 children (37.5%) one enteropathogen, in 202 children (24.6%) two enteropathogens, in 69 children (8.4%) three enteropathogens and in 19 children (2.2%) four or more enteropathogens were detected. Of 290 children with at least two enteropathogens, 219 (75.5%) had at least one virus, 191 (65.9%) had a bacterium and 178 (61.4%) had a parasite (Table 2). Most children with four or more enteropathogens were female (15/19, 78.9%) and all except one attended day-care (18/19, 94.7%). After adjusting for shared exposures, the observed frequency of co-infection was significantly higher than expected for norovirus GI and norovirus GII (expected 0.30% versus observed 0.76%, p=0.016), *C. difficile* and norovirus GI (expected 0.34% versus observed 1.16%, p<0.001), *C. difficile* and rotavirus (expected 0.14% versus observed 0.47%, p=0.011), astrovirus and *D. fragilis* (expected 0.48% versus observed 1.50%, p<0.001), atypical EPEC (a-EPEC) and adenovirus (expected 7.0% versus observed 8.8%, p=0.046), typical EPEC (t-EPEC) and adenovirus (expected 1.1% versus observed 2.5%, p<0.001) and enteroaggregative *E. coli* and astrovirus (expected 0.08% versus expected 0.31%, p=0.020) (Table 3). The frequency of co-infection was not significantly lower than expected after adjusting for shared exposures. The same co-infection patterns were observed in the PCA (see Supplementary material, Fig. S1).

Table 2. Number of single infections and co-infections of enteropathogens in children aged 0-48 months, and the percentage of children with single infections that experienced GE in the two weeks prior to stool sampling (n=822).

	Total detections	% GE	Single occurrence	Viral co-infection	Bacterial co-infection	Parasitic co-infection	Norovirus GI	Norovirus GII	Sapovirus	Astrovirus	Rotavirus	Adenovirus	Adenovirus 41	*Yersinia*	*Campylobact*	*C. difficile*	STEC	a-EPEC	t-EPEC	EAEC	*D. fragilis*	*G. lamblia*
Virus																						
Nororivus GI	38	24	7	16	26	16	-	6	1	1		12				6		14	3	3	14	4
Norovirus GII	57	44	9	28	26	22	6	-	1	2	2	25	1			7	1	18	3	2	22	2
Sapovirus	34	18	16	12	0	8	1	1	-	1	3	9				3		3			8	
Astrovirus	14	50	4	4	5	8	1	2	1	-		3						2		4	8	1
Rotavirus	12	33	2	7	3	2		2	3		-	5				3					2	
Adenovirus	228	25	65	45	103	76	12	25	9	3	5	-		1		27	8	75	16	10	74	10
Adenovirus type 41	6	67	3	1	2	1		1					-			1	1	1			1	1
Bacteria																						
Yersinia entero.	1	0	0	1	0	0						1		-							1	
Campylobacter	2	100	1	0	0	1															1	
C. difficile	69	23	26	37	14	3	6	7	3		3	27	1			-	2	12	5	3	3	
Salmonella	0	-	-	-	-	-																
Shigella spp./EIEC	0	-	-	-	-	-																
STEC (*stx* 1/2)	23	13	2	8	2[a]			1				8	1				-	18		2	8	
a-EPEC (*escV*)	195	22	41	93	12[a]		14	18	3	2		75	1			12	18	-	32	10	69	11
t-EPEC (*bfpA*)	32	34	0	17	5[a]		3	3				16				5		32	-	3	14	3
EAEC (*aggR*)	41	24	11	14	3[a]		3	2		4		10				3	2	10	3	-	19	3
Parasite																						
D. fragilis	272	20	101	109	90	27	14	22	8	8	2	74	1	1	1	3	8	69	14	19	-	27
G. lamblia	39	13	5	14	14	27	4	2		1		10	1					11	3	3	27	-
E. histolytica	0	-	-	-	-	-																
Cryptosporidium	1	100	1	-	-	-																

[a]Excluding co-infections between pathogenicity genes *stx 1/2* (STEC), *escV* (a-EPEC), *bfpA* (t-EPEC), *aggR* (EAEC)

Table 3. Observed and expected percent prevalence of co-infection of each enteropathogen with the other enteropathogens, with and without adjusting for shared exposure (n=822). Two-sample Z-tests for proportions were used to compare the observed and expected percent prevalence; red ($p<0.01$), orange ($p<0.05$), yellow ($p<0.10$), grey ($p\geq0.10$). Only those with a p-value <0.10 are shown.

		Norovirus GI	Norovirus GII	Sapovirus	Astrovirus	Rotavirus	Adenovirus	*C. difficile*	STEC	a-EPEC	t-EPEC	EAEC	*D. fragilis*	*G. lamblia*
Norovirus GI	Expected %		0.30					0.34						0.22
	Observed %		0.73					0.73						0.49
Norovirus GII	Expected %	0.30					1.98							
	Observed %	0.76[a]					3.04							
Sapovirus	Expected %					0.08				1.06				
	Observed %					0.36				0.36				
Astrovirus	Expected %											0.08	0.48	
	Observed %											0.49	0.97	
Rotavirus	Expected %			0.08				0.14		0.39				
	Observed %			0.19[b]				0.36		0.0				
Adenovirus	Expected %									7.0	1.1			
	Observed %									9.1	2.0			
C. difficile	Expected %	0.34				0.14							1.45	
	Observed %	1.16[c]				0.47[d]							0.36	

Table 3. Continued.

		Norovirus GI	Norovirus GII	Sapovirus	Astrovirus	Rotavirus	Adenovirus	*C. difficile*	STEC	a-EPEC	t-EPEC	EAEC	*D. fragilis*	*G. lamblia*
STEC	Expected %													
	Observed %													
a-EPEC	Expected %			1.06		0.39	7.0							
	Observed %			0.54[e]		0.0	8.8[f]							
t-EPEC	Expected %						1.1							
	Observed %						2.5[g]							
EAEC	Expected %				0.08									
	Observed %				0.31[h]									
D. fragilis	Expected %				0.48			1.45						1.82
	Observed %				1.39[i]			0.82[j]						3.28
G. lamblia	Expected %	0.22											1.82	
	Observed %	0.48[k]											2.37[l]	

The observed prevalence was adjusted for age[a-f, i-k], gender[a,c,k] season[a,b,d-g,i], day-care attendance[a,b,c,k,l], urbanization degree[a,c,h-k], number of children in the household[c,d,h,i,j,l], presence of farm animals[a,c,k] or cats[k,l], or dogs[i,j], or sandpits[k,l] in the household, presence of horses, pony's or donkey's in the household or in the day-care center[h]

Gastrointestinal illness

In children with no enteropathogens detected, 50/224 (22.3%) had GE, compared with 68/308 (22.1%, χ2 test, p=0.947) in children with one enteropathogen, 43/202 (21.3%, χ2 test, p=0.796) in children with two enteropathogens, and 24/88 (27.3%, χ2 test, p=0.309) in children with three or more enteropathogens, but differences were not significant. Co-infections of adenovirus and norovirus GII, and of a-EPEC with norovirus GII, were significantly associated with GE (12/25, 48.0%; OR 3.0, 95%CI 1.3-7.1 and 8/18, 44.4%; OR 2.7, 95%CI 1.0-7.3, respectively), compared with children with no enteropathogens detected (Table 4). This was probably due to the increased risk for GE of norovirus GII alone, because neither co-infection was associated with GE when compared with children with norovirus GII alone (OR 1.3, 95%CI 0.4-3.6 and OR 1.0, 95%CI 0.3-3.1, respectively).

Table 4. Multivariable logistic regression model of the association between gastroenteritis and combinations of enteropathogens, compared with children that were negative for all enteropathogens that were tested. Here, only models are shown where the combination of enteropathogens were significantly associated (p<0.05) with gastroenteritis.

	N	%GE[b]	aOR[c]	95%CI[d]	p-value
Adenovirus x norovirus GII					
None[a]	244	22	Ref		
Adenovirus	203	22	0.9	0.6-1.4	0.616
Norovirus GII	32	41	2.4	1.1-5.2	0.029
Adenovirus x norovirus GII	25	48	3.0	1.3-7.1	0.011
a-EPEC x norovirus GII					
None[a]	244	22	Ref		
a-EPEC	177	19	0.8	0.5-1.3	0.350
Norovirus GII	39	44	2.7	1.3-5.6	0.006
a-EPEC x norovirus GII	18	44	2.7	1.0-7.3	0.048

[a]Negative for all enteropathogens that were tested; [b]Gastroenteritis; [c]Odds ratio, adjusted for underlying enteropathies and age; [d]95% confidence interval

Risk factors for co-infection

Having two or more enteropathogens compared with a single enteropathogen was associated with being <12 months old (OR 1.9, 95%CI 1.1-3.3) or 13-36 months old (OR 1.7, 95%CI 1.1-2.5), compared with being 37-48 months old, day-care attendance (OR 1.8, 95%CI 1.3-2.5) and living in a household with at least three children compared to living in a household with one child (OR 1.7, 95%CI 1.1-2.8) (Table

5). Stool samples taken during spring less often had two or more enteropathogens compared with those taken in summer (OR 0.4, 95%CI 0.2-0.7).

Having three or more enteropathogens compared with a single enteropathogen was associated with having a food allergy (OR 3.2, 95%CI 1.1-8.9) and day-care attendance (OR 2.0, 95%CI 1.2-3.4). Stool samples taken during spring were less likely to have three or more enteropathogens compared with those taken during summer (OR 0.3, 95%CI 0.1-0.7)

Table 5. Multivariable logistic regression model of risk factors in children with more than one enteropathogen and more than two enteropathogens compared with children with one enteropathogen

	1 enteropathogen n=308		**>1 enteropathogen** n=290				**>2 enteropathogens** n=88			
	N	**%**	**N**	**%**	**aOR[a]**	**95%CI[b]**	**N**	**%**	**aOR[a]**	**95%CI[b]**
Child's age										
37-48 months	95	30.8	67	23.1	Ref		16	18.2	Ref	
13-36 months	170	55.2	179	61.7	**1.7**	**1.1-2.5**	59	67.1	**2.2**	**1.2-4.1**
≤12 months	43	14.0	44	15.2	**1.9**	**1.1-3.3**	13	14.8	**2.5**	**1.0-5.8**
Food allergy	9	2.9	16	5.5	ns	-	8	9.1	**3.2**	**1.1-8.9**
Day-care attendance	165	53.8	187	64.5	**1.8**	**1.3-2.5**	60	68.1	**2.0**	**1.2-3.4**
No. children in the household										
1	91	29.6	77	26.6	Ref		23	26.1	Ref	
2	148	48.2	134	46.2	1.1	0.8-1.7	42	47.7	ns	-
≥3	68	22.2	79	27.2	**1.7**	**1.1-2.8**	23	26.1	ns	-
Season										
Summer	115	37.3	130	44.8	Ref		43	48.9	Ref	
Autumn	65	21.1	55	19.0	0.7	0.5-1.2	21	23.9	0.8	0.4-1.6
Winter	62	20.1	73	25.0	1.0	0.7-1.6	15	17.1	0.6	0.3-1.2
Spring	66	21.4	32	11.0	**0.4**	**0.2-0.7**	9	10.2	**0.3**	**0.1-0.7**

ns=not significant (p<0.05) and therefore not included in the model; [a]Adjusted Odds Ratio; [b]95% Confidence Interval

Discussion

This study identified several risk factors for co-infection in preschool children. Co-infections were detected in 290 (48.5%) of 598 children who had at least one enteropathogen, but were not associated with increased GE risk. We also observed

that several enteropathogens co-occurred more frequently than would be expected by chance.

Norovirus GI and norovirus GII co-infection occurred more often than expected. This could be due to their largely overlapping epidemiology, even when adjusting for shared exposures. Another explanation may be that around 20% of Dutch inhabitants do not have the FUT2 allele required for histo-blood group antigen expression at the gut surface, to which norovirus binds (18, 19). Therefore, they have near-total protection against some norovirus genotypes, including the dominant norovirus GII.4 in the Netherlands (18, 20-23). Hence, norovirus GI and GII co-infection may not occur randomly but more often in histo-blood group antigen secretors. However, other molecules and cell types binding to norovirus may also play a role, but are not well understood (19). Co-infection of *C. difficile* with norovirus GI and rotavirus also occurred more frequently than expected. A US study reported higher fecal *C. difficile* ribotype 027 concentrations in children with viral co-infections compared with children without co-infections (24). They stated that viruses may create favorable conditions for *C. difficile* to multiply, possibly through disruption of the intestinal microbiota or host defenses, which was also hypothesized in two case reports (25, 26). However, evidence is scarce, and ribotyping was not performed in our study, questioning the comparability with the US study. Co-infections of astrovirus with enteroaggregative *E. coli* and *D. fragilis*, and adenovirus with t-EPEC and a-EPEC, were found more often than expected. To our knowledge, there is no literature describing possible interactions between them. Previous research based on the same study population found that astrovirus was significantly associated with GE and t-EPEC, which pointed towards an association, albeit non-significant (11). We speculate that GE might flush out other enteropathogens as well, increasing their detection chance, possibly explaining why these co-infections were observed more than expected.

Only co-infection of norovirus GII with adenovirus and norovirus GII with a-EPEC were associated with GE. However, this was probably a result of increased risk for GE of norovirus GII alone (11). Indeed, when comparing both co-infections with norovirus GII single infections, no increased GE risk was observed. Comparing our results with other studies is difficult because they mostly focus on children admitted to hospital and can therefore only examine GE severity (3, 6-9, 27, 28). However, they show conflicting results regarding the clinical relevance of co-infections. Two studies did compare children with and without GE. A study in Mexico compared children hospitalized for GE with children without GE from

surrounding schools, and found that co-infections were more prevalent in those hospitalized (29). However, they only associated co-infection as a whole with GE and not specific enteropathogens, and focused on a different panel of enteropathogens. Co-infections were not associated with GE in a Korean study comparing hospitalized children with and without GE, but did aggravate GE symptoms (30). We were unable to assess GE severity because no data were collected on duration and frequency of symptoms, which are the most common criteria to define GE severity.

Younger children more often had co-infections, which could be because almost all co-infections included at least one virus, which were more prevalent in younger children, as were bacteria (11). Furthermore, da-care-attending children were more often co-infected, probably reflecting increased enteropathogen exposure in the day-care centre compared with those children who were home-cared (11). Co-infections were also more prevalent in children living in households with two or more children compared with children being the only child, probably reflecting increased transmission between children (31). We observed that stool samples collected during spring had fewer co-infections than those in summer, probably because viruses were mostly detected during autumn and winter and bacteria and parasites during autumn (11). Lastly, children with a food allergy had more co-infections with three or more enteropathogens. Children with food allergy may be more likely to have gastrointestinal symptoms, leading to a disturbed normal gut flora and thereby increased vulnerability to infections (32). Moreover, an immature mucosal immune system has been described as a risk factor for food allergy, likely also increasing the chance for co-infections (32).

This study has several limitations. Due to its cross-sectional design, the chronological order of the infections caused by the different enteropathogens involved was unknown. This makes it more difficult to understand the underlying mechanism(s) of possible enteropathogen interactions. Furthermore, although the current study only focussed on enteropathogens, complex relationships between enteropathogens and non-pathogenic microorganisms probably also influence the outcome of infection. When assessing the effect of co-infection on GE, children with no enteropathogens detected were the reference group, possibly making the results less generalizable to areas with different testing capabilities. However, results were identical when single infections of both enteropathogens in question were the reference groups (results not shown). Because symptoms in children were self-reported by their parents, misclassification of GE may have occurred due to diarrhoea or vomiting that occurred as a result of non-infectious causes.

Furthermore, the presence of enteropathogens in stool was determined based on the detection of genetic material. This could have led to an overestimation, as this can be detected in faeces up to several weeks after infection (33). Lastly, although we adjusted for shared exposure when analysing whether co-infections occurred more or less often than expected, it is unlikely that we were able to fully adjust for it.

In conclusion, we observed that several enteropathogens co-occur the guts of preschool children non-randomly and independently of exposure to common risk factors. We hypothesize possible enteropathogen interactions that would need to be tested further, for example in experimental settings. Co-infections were associated with crowding in households, use of day-care centres and younger age. Yet, co-infections did not lead to a significantly increased GE risk in our study population, questioning the clinical relevance of such co-infections in preschool children in industrialized countries.

Supporting material

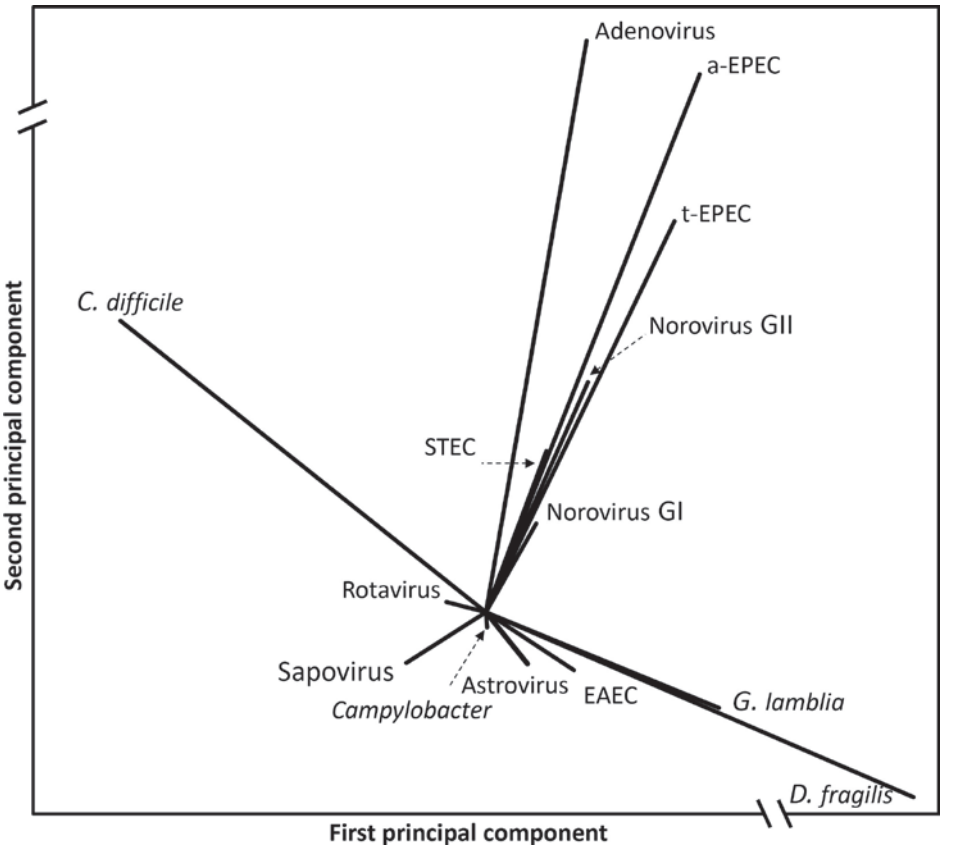

Figure S1. First two principal components of the principal component analysis (PCA) visualizing correlations between enteropathogens. The first principal component explains 22.5% of the variance in the data and the second principal component explains 21.8% of the variance. If enteropathogens in a component are positively correlated with each other, loadings will be positive (i.e. they will "point" in the same direction), vice versa.

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CHAPTER 6

Characteristics of child daycare centres associated with clustering of major enteropathogens

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Published in Epidemiology and Infection, 2016

Abstract

Background

Insights into transmission dynamics of enteropathogens in children attending daycare are limited. Here we aimed at identifying daycare centre (DCC) characteristics associated with time clustered occurrence of enteropathogens in DCC-attending children.

Methods

We used data from the KIzSS network, which comprises 43 DCCs that participated in infectious disease surveillance in The Netherlands during February 2010–February 2013. Space-time scan statistics were used to identify clusters of rotavirus, norovirus, astrovirus, *Giardia lamblia* and *Cryptosporidium* spp. in a two-dimensional DCC characteristic space constructed using canonical correlation analysis. Logistic regression models were then used to further identify DCC characteristics associated with increased or decreased odds for clustering of enteropathogens.

Results

Factors associated with increased odds for enteropathogen clustering in DCCs were having indoor/outdoor paddling pools or sandpits, owning animals, high numbers of attending children, and reporting outbreaks to local health authorities. Factors associated with decreased odds for enteropathogen clustering in DCCs were cleaning child potties in designated waste disposal stations, cleaning vomit with chlorine-based products, daily cleaning of toys, extra cleaning of toys during a suspected outbreak, and excluding children with gastroenteritis.

Conclusions

These factors provide targets for reducing the burden of gastrointestinal morbidity associated with time clustered occurrence of major enteropathogens in DCC attendees.

Introduction

More than half of preschool children in The Netherlands (<4 years old) experience at least one gastroenteritis (GE) episode each year [1]. About 40% of children in this age group are cared for in day-care centres (DCCs), where they are at increased risk for GE and infectious diseases in general [2-4]. A recent Dutch study reported that these children are almost twice as likely to experience GE needing medical attention as expected based on national estimates [5]. This is comparable with other studies from the United States, reporting out-of-home childcare to be associated with a relative risk for diarrhoeal illness in preschool children of 1.8 compared to home care [6]. Although most of these GE episodes are mild and self-limiting, they may have an impact on parents/caregivers and on the society as a whole via productivity losses (e.g. days of work lost for care) and increased expenditure on healthcare services and treatment [1, 7]. Decreasing gastrointestinal morbidity in children attending DCCs requires insights into transmission dynamics and targets for control, which are currently not well established.

A recent Dutch study has identified several socio-demographic, facility- and policy-related DCC characteristics associated with GE incidence as a whole and with the prevalence of specific enteropathogens in DCC attendees [8]. However, this study did not distinguish between sporadic and clustered occurrence of these enteropathogens, while their risk factors may well differ. Space-time scan statistics have been applied to investigate clustering of infectious agents in hospitals and other community care services [9-12], and may also help identify clustering of enteropathogens in DCC-attending children and risk factors involved.

In this study, we exploit projections of DCCs in a two-dimensional space based on similarities of DCC characteristics, making it possible to apply space-time scan statistics to detect temporal clusters of pathogen detections in DCC attendees in relation to DCC characteristics. Clusters of pathogen occurrence are defined here as non-trivial groupings of individual pathogen detections as revealed by 'expectation-based' scan statistics. These statistics fit baseline models to the observed data in order to identify where and when there have been anomalous numbers of pathogen detections given those expected. Clusters can present themselves in different forms. They can have varying time spans and can either be restricted to small areas (i.e. associated with one or a few DCC characteristics) or be widespread (i.e. associated with many DCC characteristics). Outbreaks, seasonal and non-seasonal increases in pathogen circulation may all result in clustering. Although an increase in pathogen

occurrence may mirror an increase in pathogen activity, it does not necessarily mean an excess of disease burden, as an infection can also be asymptomatic. While seasonality usually results in periodic (year-to-year) clustering, outbreaks and non-seasonal increases are more likely to manifest themselves irregularly. Investigating pathogen clusters using scan statistics may therefore embrace several potential outcomes, offering the opportunity of generating novel epidemiological insights towards understanding a pathogen's temporal pattern and risk factors involved.

The aims of this study were to: 1) assess potential clustering of five major childhood enteropathogens (rotavirus, norovirus, astrovirus, *Giardia lamblia* and *Cryptosporidium* spp. [8]) in time as related to DCC characteristics; and 2) identify which socio-demographic, facility- and policy-related DCC characteristics were those associated with the clustered occurrence of these enteropathogens in DCC attendees; and 3) reveal possible differences with DCC characteristics associated with pathogen prevalence as a whole.

Materials and Methods

Data sources

We used microbiological and epidemiological data from the KIzSS network, which comprises DCCs that participated in infectious disease surveillance in The Netherlands during 2010–2013 [13]. In March 2010, 2011 and 2012, all DCCs registered in the Dutch national database maintained by the Ministry of Social Affairs and Employment in The Netherlands were invited to participate in a dynamic DCC network for the surveillance of enteropathogens in their child populations. A detailed description of the DCC network design, methodology, definitions and limitations has been reported elsewhere [8, 13].

Forty-three DCCs agreed to submit 10 randomly selected faecal samples on a monthly basis from their attending children aged up to 48 months, regardless of the presence of gastrointestinal symptoms at the time of sampling. Of these, only 36 DCCs actually submitted samples (Fig. 1). Only one faecal sample per child within the same month was allowed to be submitted. All faecal samples were tested for 16 different enteropathogens using multiple, internally controlled quantitative PCRs (Table 1) [13]. For detailed information about the molecular detections methods, we refer to a previously published open-access paper [1]. This study focused on five major enteropathogens that were significantly associated with GE incidence in

DCC attendees [14], accounting for 39% of GE morbidity; 11% by rotavirus, 10% by norovirus, 8% by *G. lamblia*, 7% by astrovirus, and 3% by *Cryptosporidium* spp. [14].

At the time of recruitment, DCCs were asked to complete a questionnaire on their characteristics, covering three main categories: socio-demographics, facility design and policies for hygiene and disease control, of which a detailed description is reported elsewhere [8]. This study builds upon and uses the same data of a previous study [8] to which we refer for more information. While the previous study looked at associations between DCC characteristics and enteropathogen prevalence as a whole, the present study looked at associations between DCC characteristics and clustered occurrence of these enteropathogens. Thus, the two studies differ in the outcome of interest and in the approach used. For the purpose of this study, we focused on DCC characteristics that were found to be significantly associated with the prevalence of rotavirus, norovirus, astrovirus, *G. lamblia* or *Cryptosporidium* spp. in DCC attendees in the previous study (Table 2) [8].

Reconstructing the 'spatial' distribution of DCC characteristics

Ideally, the true geographical locations of the DCCs would be used to identify space-time clusters of enteropathogens to infer physical transmission of enteropathogens among DCCs. However, since the 43 DCCs of this study were scattered all over The Netherlands (Fig. 1), any cluster incorporating multiple DCCs was unlikely to be the result of direct enteropathogens transmission between DCCs, as they were located too far apart from each other to be physically related to one another. Therefore, we looked at temporal clustering of enteropathogens over infrastructural similarities, rather than geographical proximities of the DCCs. To this end, we constructed an alternative spatial distribution for the participating DCCs using canonical correlation analysis (CCA). CCA is a statistical method that finds the multivariate relation(s) between two sets of variables (DCC characteristics in one set and enteropathogens in the other), with each set consisting of two or more variables [15]. CCA was used here to calculate linear combinations of DCC characteristics (so called canonical variates) that maximize relationships to a similarly calculated linear combination of enteropathogens. This means that DCCs were positioned in the reconstructed space according to their groupings of common characteristics, with those DCCs positioned closer to each other in the reconstructed space being those more similar to one another with regard to their characteristics putatively associated with enteropathogen occurrence, with the opposite applying for those DCCs that were placed far apart in the reconstructed space (Fig. 2). This alternative space, where the DCCs were place, was defined by the first and second canonical variates, hereafter referred to as DCC-characteristic space (Fig. 3).

Figure 1. Geographical location of the 36 day-care centres, represented by their ID numbers, included in this study, the Netherlands.

Table 1. Enteropathogens included in the microbiological surveillance

Virus	Bacteria	Parasites
Norovirus[1]	*Escherichia coli*[3]	*Giardia lamblia*[1]
Sapovirus	*Salmonella enterica*	*Cryptosporidium* spp.[1]
Rotavirus[1]	Shigella spp.	*Dientamoeba fragilis*
Adenovirus[2]	*Campylobacter jejuni*	
Astrovirus[1]	*Clostridium difficile*	
	Yersinia enterocolitica	

[1]The current study focused on these five enteropathogens; [2]Enteropathogenic types 30 and 40/41; [3]Shiga toxin producing *Escherichia coli* (STEC), enteroaggregative *E. coli* (EAEC) and typical and atypical enteropathogenic *E. coli* (EPEC)

Cluster analysis

The series of faecal samples submitted between February 2010 and February 2013 were analysed retrospectively for the presence of rotavirus, norovirus, astrovirus, *G. lamblia* and *Cryptospordium* spp. temporal clusters in the DCC-characteristic space. Analyses were performed using month-level Bernoulli models in SaTScan v. 9.1.1 [16]. The maximum temporal window for a cluster was set to 50% of the study period (18 months) and a maximum of 50% of the study population was allowed to be included in a cluster. The scan statistic is defined by a cylindrical window varying in size, with a circular spatial base and with height corresponding to time, which evaluates each possible time period, centring the window on each DCC in the DCC-characteristic space. For each DCC, only the cylinder with the maximum likelihood is reported as the most likely cluster, which is calculated based on the number of observed and expected number of pathogens inside and outside the cylinder. A *P* value is assigned via 999 Monte Carlo simulations [17]. Scan statistics were performed on six temporally contiguous time windows of 6 months each, from February 2010 to January 2013. In addition, six additional temporally contiguous time windows were analysed with a 6-month interval, from May 2010 to February 2013. The clusters so identified, represented increased occurrences in time of enteropathogen-positive faecal samples in children attending DCCs with similar characteristics.

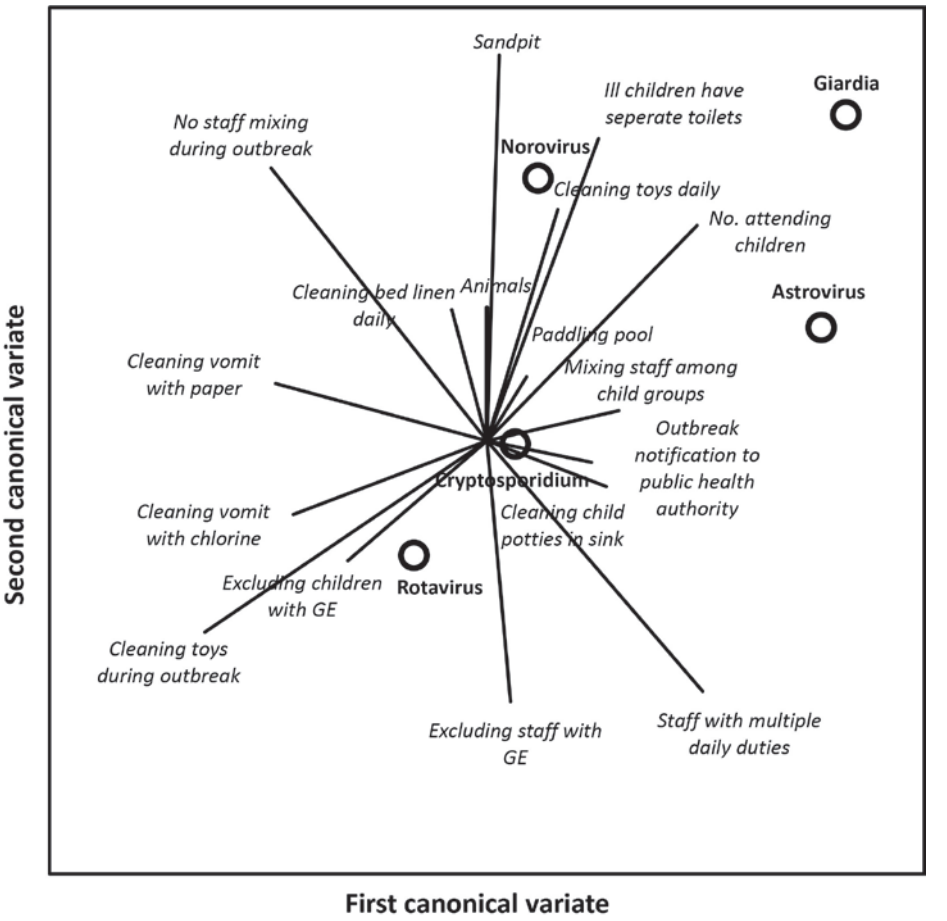

Figure 2. Plot of the DCC characteristics and enteropathogens over the first and second canonical variate, i.e. the two main dimensions of the DCC characteristics space (cf. Figure 3).

Analysis of DCC characteristics associated with clustering

Clusters identified using SaTScan with a $P > 0.05$ by the likelihood ratio test were discarded. Overlapping clusters in time and DCC were merged for each pathogen. DCCs were excluded from a cluster when/if they did not send any samples during the time span of the cluster. Based on the results of the cluster analyses, a dataset was constructed in which each DCC was either included in a cluster (encoded as 1) or not (encoded as 0) at each month of the study period, thereby obtaining a binary dataset of 1332 DCC-month observations (i.e. 36 DCCs followed-up for 37 months) for each pathogen. Pathogen presence or absence was assigned to a month based on the first day of the week the faecal sample was collected.

We then assessed whether the aforementioned 21 DCC characteristics were significantly associated with a higher or lower odds for a DCC to be included in a space-time cluster (i.e. those identified using SatScan) of rotavirus, norovirus, astrovirus, *G. lamblia* or *Cryptospordium* spp. using logistic regression. A separate analysis was performed for each pathogen. Associations of the 17 DCC characteristics listed in Table 2 were first assessed one by one in a baseline model including socio-economic status (expressed as a normalized score from -4 to +4 based on income, employment and educational level per postal code area, with a higher score indicating lower socioeconomic status), urbanization degree [categorized as 'highly urbanized' (>2500 addresses/km^2), 'urbanized' (1500–2500 addresses/km^2), 'moderately urbanized' (1000–1500 addresses/km^2), 'lowly urbanized' (500–1000 addresses/km^2), and 'rural' (<500 addresses/km^2)], season [categorized as 'winter' (December–February). 'spring' (March–May), 'summer' (June–August), 'autumn' (September–November)] and calendar year (2010 to 2013) as control variables. DCC characteristics with a $P \leq 0.10$ at the single-variable analysis were then entered in a multivariable logistic regression model built in backward stepwise fashion: only variables with a p-value <0.05 were retained. The effect of removing variables from the model on the coefficients of other variables was also monitored. Collinearities between independent variables were checked prior to regression analysis with cross-tabulations and collinear variables were selected based on improved model fit (Akaike information criterion) or combined in one variable when they predicted each other perfectly. Multivariable logistic regression models with an overall statistical significance (likelihood ratio χ^2 test, $P < 0.05$) and goodness-of-fit (Hosmer–Lemeshow test, $P > 0.05$) are reported. Seasonal and non-seasonal clusters were distinguished in order to identify whether risk factors might differ. Clusters occurring in the same season for three subsequent years were classified as seasonal, and non-seasonal otherwise. Statistical analyses were performed using Stata v. 13.0 software (StataCorp, USA).

Results

From February 2010 to February 2013, 5125 faecal samples were submitted by the 36 enrolled DCCs. Of these samples, 148 (2.9%) tested positive for astrovirus, 478 (9.3%) for norovirus, 153 (3.0%) for rotavirus, 39 (0.8%) for *Cryptospordium* spp. and 219 (4.3%) for *G. lamblia*.

Clustering of enteropathogens

A total of 229 clusters over all five enteropathogens were identified during the study period (Fig. 4). For rotavirus, 79 clusters were found in 35 different DCCs (97% of all DCCs participating), including 82.4% of all samples found positive for rotavirus, with a time span of 1–5 months (median 2 months). For norovirus, 76 clusters were found in 27 DCCs (75%), including 77.8% of all samples found positive for norovirus, with a time span of 1–12 months (median 2 months). For astrovirus, 43 clusters were found in 31 DCCs (86%), including 70.9% of all samples found positive for astrovirus, with a time span of 1–4 months (median 2 months). For *G. lamblia*, 29 clusters were found in 15 DCCs (42%), including 53% of all samples found positive for *G. lamblia*, with a time span of 1–11 months (median 3 months). For *Cryptospordium* spp., 22 clusters were found in 18 DCCs (50%), including 79.5% of samples found positive for *C. hominis*, with a time span of 1–5 months (median 2 months).

Table 2. DCC characteristics focussed on in this study that were significantly associated with the prevalence of rotavirus, norovirus, astrovirus, *G. lamblia* or *Cryptosporidium* spp. among DCC attendees in a previous study based on the KIzSS network [8].

Hygiene policies	**Control measures during suspected outbreak**
Cleaning vomit with paper[1]	Excluding children with GE
Cleaning vomit with chlorine-based products	Excluding personnel with GE
Cleaning toys more or less frequently than daily	No mixing of staff members among child groups
Cleaning bed linen more or less frequently than daily	Extra cleaning of toys
Cleaning child potties in a normal sink[2]	Notification to local health authorities
Facility design	**Demographics**
Presence of dedicated toilets for ill children	Weekly number of attending children
Presence of animals	Mixing of personnel among child groups
Presence of paddling pools	
Presence of sandpits	
Staff members have multiple daily duties	

[1]Rather than cloth towels but without cleaner; [2]Rather than in a designated waste disposal station

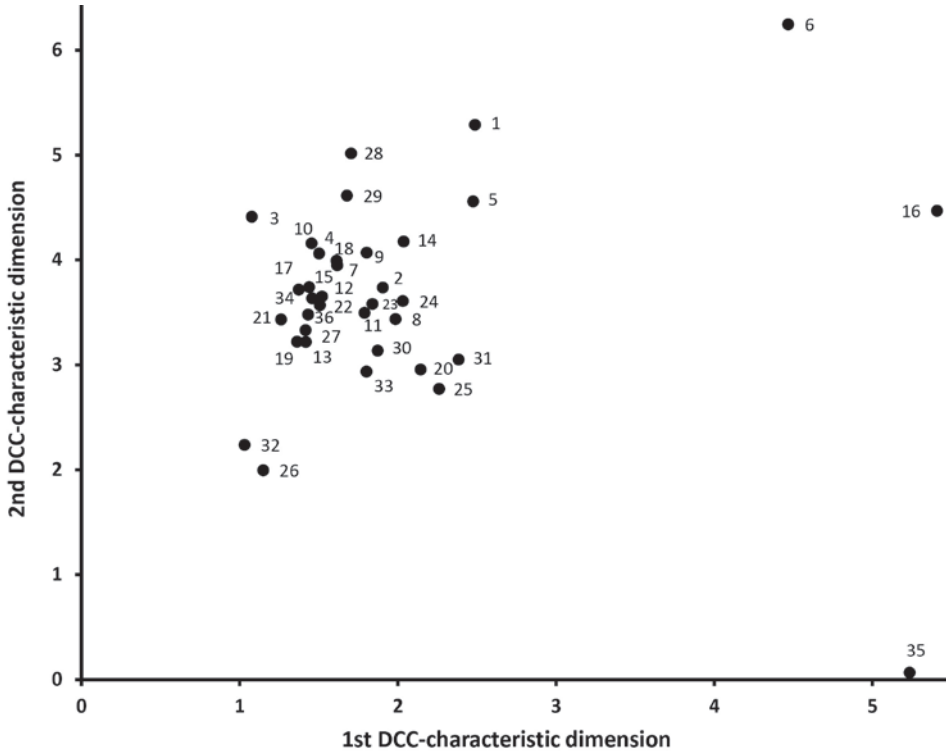

Figure 3. Reconstructed spatial distribution in the DCC-characteristics space of the 36 DCCs included in this study, represented by their ID numbers.

All clusters of rotavirus and astrovirus were classifiable as seasonal, whereas those of norovirus, *G. lamblia*, and *Cryptosporidium* spp. were classifiable as non-seasonal (Fig. 4). Therefore, we did not stratify our risk factor analysis by seasonal and non-seasonal clustering since all clusters for a given pathogen appeared to be of the same type. Seasonal clusters were observed during the whole study period for rotavirus, and from 2011 to 2013 for astrovirus. For norovirus, *G. lamblia* and *Cryptospordium* spp. non-seasonal clusters were identified throughout the year. No clusters were found after January 2012 for norovirus, while clustered *Cryptospordium* spp. occurrence was observed only in 2012. *G. lamblia* clusters were found throughout the study period, with a longer time span and fewer DCCs involved, compared to the other enteropathogens.

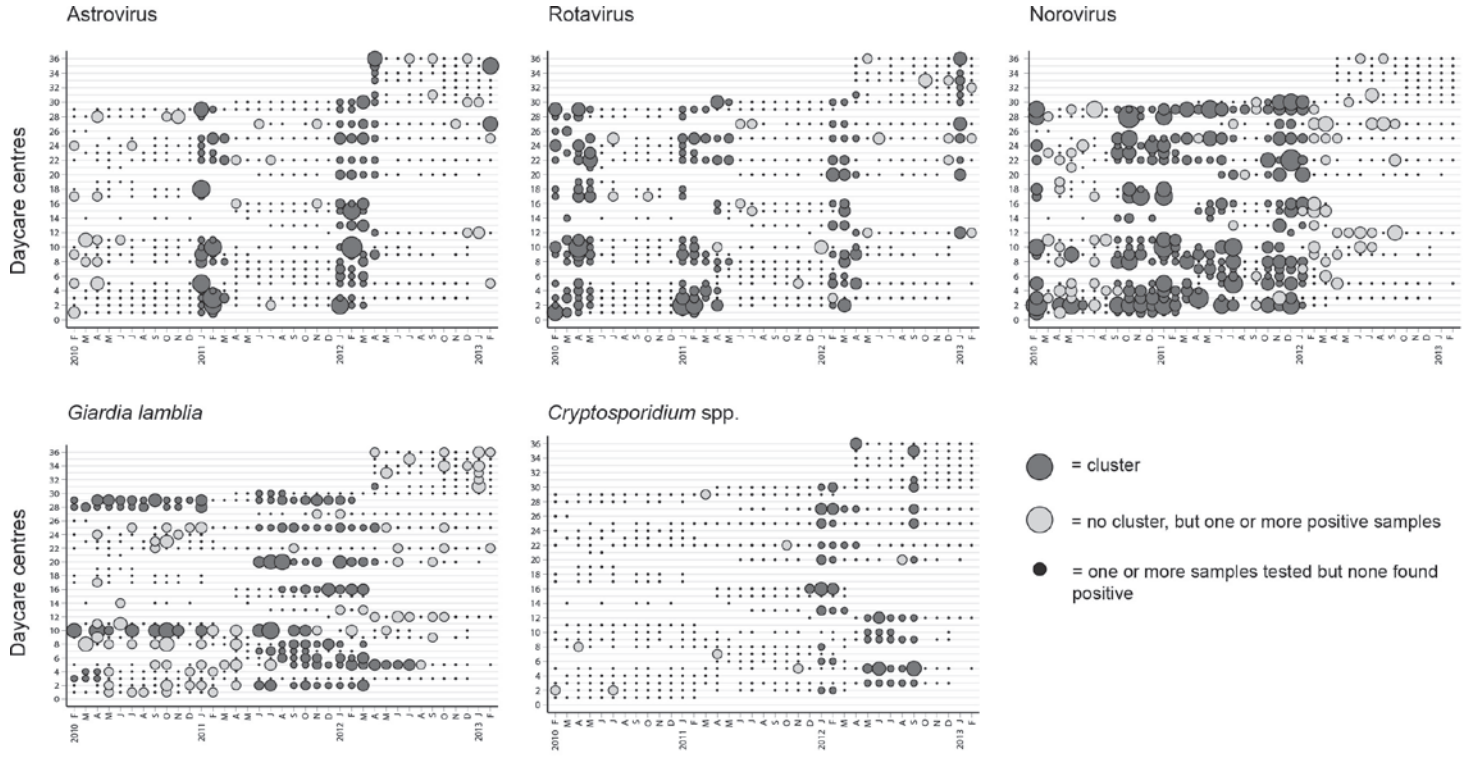

Figure 4. Clusters of astrovirus, rotavirus, norovirus, *G. lamblia* and *Cryptosporidium* spp. in Dutch day-care centres from February 2012 to February 2013. The size of the circle correlates with the number of faecal samples positive for the relevant pathogen; no circle indicates absence of samples submitted.

DCC characteristics associated with clustering in time of *G. lamblia*

As shown in Table 3, five DCC characteristics were significantly associated with clustering of *G. lamblia* in DCCs. Keeping animals and having sandpits in the DCC was associated with an increased risk for DCCs to experience clustered occurrence of *G. lamblia* in their child populations [odds ratio (OR) 17.0, 95% confidence interval (CI) 1.7–169.4]. Reporting any suspected GE outbreak to local health authorities was also associated with a higher risk of experiencing a cluster of *G. lamblia* occurrence (OR 4.1, 95% CI 1.7–9.5), so was the presence of paddling pools (OR 2.0, 95% CI 1.2–3.6). Cleaning vomit with chlorine-based products was associated with a decreased risk for DCCs having a *G. lamblia* cluster (OR 0.3, 95% CI 0.1–0.8). The same was found for extra cleaning of toys during a suspected GE outbreak (OR 0.3, 95% CI 0.1–0.6).

DCC characteristics associated with clustering in time of *Cryptospordium* spp.

Two DCC characteristics were associated with clustering of *Cryptospordium* spp. in DCCs. Cleaning toys daily compared to cleaning toys less than daily and excluding children with GE from entering the DCC were both associated with a decreased risk for DCCs to experience clustered occurrence of *Cryptospordium* spp. in their attendees (OR 0.3, 95% CI 0.1–0.9 and OR 0.1, 95% CI 0.0–0.4, respectively).

DCC characteristics associated with clustering in time of rotavirus and astrovirus

Only the presence of sandpits in the DCC was found to be associated with clustering of rotavirus in DCCs (OR 4.0, 95% CI 1.2–13.1). The same association was found for astrovirus (OR 3.9, 95% CI 1.2–13.2).

DCC characteristics associated with clustering in time of norovirus

Five DCC characteristics were associated with clustering of norovirus in DCCs. A higher number of DCC attendees was associated with increased risk for DCCs to experience a cluster of norovirus occurrence (OR 2.4, 95% CI 1.6–3.6). DCCs with sandpits or paddling pool were at increased risk of experiencing clustered occurrence of norovirus in their attendees (OR 5.5, 95% CI 2.7–11.3 and OR 4.3, 95% CI 2.3–8.2, respectively). Cleaning child potties in a dedicated sink was associated with decreased risk of having norovirus clusters (OR 0.4, 95% CI 0.2–0.7), so was cleaning of toys during a suspected outbreak (OR 0.5, 95% CI 0.3–0.9).

Table 3. Adjusted odds ratios and corresponding 95% confidence intervals for the association between day-care centre characteristics and clustering of rotavirus, norovirus, astrovirus, *G. lamblia* and *Cryptosporidium* spp. in DCCs

	G. lamblia[1]	*Cryptosporidium*[1]	Rotavirus[1]	Astrovirus[1]	Norovirus[1]
Demographics					
DCC capacity (no. attending children)	ns	ns	ns	ns	2.40 (1.61–3.56) ¶
Socioeconomic status neighborhood	2.34 (1.56 – 3.49)	0.64 (0.44 – 0.94)	0.79 (0.58 – 1.07)	0.89 (0.61 – 1.28)	1.10 (0.86 - 1.41)
Urbanization degree	Ref.	Ref.	Ref.	Ref.	Ref.
<500 addresses/km^2	0.69 (0.18 – 2.63)	0.28 (0.09 – 0.95)	1.31 (0.50 – 3.38)	2.01 (0.64 – 6.30)	4.78 (1.92 – 11.91)
500–1000 addresses/km^2	0.92 (0.34 – 2.44)	0.11 (0.03 – 0.44)	0.80 (0.31 – 2.07)	1.35 (0.44 – 4.12)	3.43 (1.51 – 7.79)
1000–1500 addresses/km^2	1.18 (0.34 – 4.03)	0.08 (0.02 – 0.39)	0.77 (0.24 – 2.48)	1.33 (0.31 – 5.71)	4.56 (1.69 – 12.28)
1500–2500 addresses/km^2	6.46 (1.51 – 27.68)	0.20 (0.05 – 0.75)	1.32 (0.40 – 4.38)	1.81 (0.45 – 7.24)	2.14 (0.82 – 5.59)
>2500 addresses/km^2					
Facility design					
Staff members with multiple daily duties	ns	ns ¶	ns	ns	ns
Mixing of staff members among child groups	ns	ns	ns ¶	ns	ns
Cleaning child potties in a designated waste disposal station	ns	ns	ns ¶	ns	0.37 (0.20–0.69)
Having indoor/outdoor sandpits	na ¶	ns	4.01 (1.23–13.08)	3.90 (1.15 – 13.23)	5.50 (2.68–11.28) ¶
Having indoor/outdoor paddling pools	2.03 (1.16–3.55)	ns	ns	ns	4.30 (2.27–8.15) ¶
Owning animals (and having sandpits[2])	16.98 (1.70–169.40) ¶	ns	ns	ns	ns
Hygiene policy					
Cleaning vomit with chlorine-based products	0.29 (0.11–0.76) ¶	ns	ns	ns	ns

Table 3. Continued.

	G. lamblia[1]	*Cryptosporidium*[1]	Rotavirus[1]	Astrovirus[1]	Norovirus[1]
Cleaning vomit with paper towels	ns	ns	ns	ns	ns ¶
Daily cleaning of bed linen	ns	ns	ns	ns	ns ¶
Daily cleaning of toys	ns	0.27 (0.08–0.92)	ns	ns ¶	ns
Extra cleaning of toys during outbreak	0.25 (0.11–0.60) ¶	ns	ns	ns	0.54 (0.31–0.94)
Excluding children with gastrointestinal symptoms	ns	0.09 (0.02–0.37)	ns ¶	ns	ns
Excluding personnel with gastrointestinal symptoms	ns ¶	ns	ns	ns	ns
Ill children have separate toilets	ns	ns	ns ¶	ns	ns
Always reporting outbreaks to local health authority	4.05 (1.72–9.54)	ns	ns	ns ¶	ns
No mixing of staff members during outbreak	ns	ns	ns	ns	ns ¶
Year	Ref.	nc[3]	Ref.	nc	ns[4]
2010	2.64 (1.46 – 4.78)		0.64 (0.30 – 1.37)	Ref.	
2011	1.36 (0.69 – 2.68)		0.18 (0.08 – 0.39)	3.30 (1.60 – 6.79)	
2012	nc		0.72 (0.21 – 2.46)	0.11 (0.02 – 0.54)	
2013					
Season	Ref.	Ref.	Ref.	Ref.	Ref.
Winter	0.77 (0.37 – 1.57)	0.62 (0.28 – 1.38)	2.59 (1.44 – 4.66)	0.26 (0.13 – 0.51)	0.20 (0.11 – 0.36)
Spring	1.07 (0.55 – 2.11)	0.78 (0.37 – 1.67)	nc	nc	0.15 (0.08 – 0.28)
Summer	1.28 (0.66 – 2.49)	0.45 (0.20 – 1.03)	nc	nc	0.78 (0.47 – 1.31)
Autumn					

ns = non-significant (p>0.05); na = non-assessable (perfect predictor); nc = non-calculable (no clusters found)

[1]Adjusted for season, urbanization degree, socio-economic status and year; [2]Owning animals perfectly predicts having sandpits; [3]Excluded from the model since no clusters were identified in 2010 and 2013, and only one cluster was identified in 2011; [4]Goodness-of-fit of the model improved with year excluded; ¶Significantly associated with pathogen prevalence as a whole in a previous study based on the same KIzSS network [8]

Discussion

This study identified several DCC characteristics that were associated with temporal clustering of rotavirus, norovirus, astrovirus, *G. lamblia* and *Cryptosporidium* spp. in DCCs. Using the same data from the KIzSS network, differences were found between DCC characteristics associated with the clustered occurrence of these enteropathogens and those previously associated with pathogen prevalence as a whole [8]. This is the first study in which a combination of different methods like multivariate, clustering and regression analyses was used to define the epidemiological pattern of clusters of these enteropathogens in DCCs and the associated risk factors.

We found enteropathogen clusters to be associated with having sandpits and paddling pools in the DCC. Specifically, DCCs with sandpits were at increased risk of experiencing clusters of rotavirus, astrovirus, norovirus and *G. lamblia*. In the previous study [8], only norovirus and *G. lamblia* were associated with increased (albeit not necessarily clustered) prevalence of norovirus and *G. lamblia* in DCC attendees in DCCs with sandpits [8]. This might suggest that DCCs with sandpits are significantly more likely to experience clustering of rotavirus and astrovirus rather than increased prevalence of these pathogens per se. Contamination of sandpits with faecal coliforms has been reported in a Japanese study in public park sandpits [18] and a documented outbreak of norovirus occurred in a nursery school as a result of a child vomiting in a sandpit [19]. For the clustered occurrence of rotavirus and astrovirus, having a sandpit was the only risk factor identified, reflecting the similarities in the epidemiology of these two viruses. Paddling pools were also associated with increased occurrence of *G. lamblia* and norovirus clusters. As all DCCs had an outdoor play area, sandpits and paddling pools as risk factors for enteropathogen clustering are unlikely to be proxies for outdoor playing, In the previous study [8], paddling pools were associated with increased prevalence of norovirus, but not with that of *G. lamblia*. Similar to sandpits, it can be speculated that the presence of paddling pools would result in increased clustering of *G. lamblia*. *G. lamblia* is known for its ability to be transmitted through contaminated water [20], and the vulnerability of swimming pools to norovirus contamination – even in the absence of any obvious vomiting or 'faecal accident' has been claimed [21].

Several hygiene-related DCC characteristics were also associated with clustering of enteropathogens. This is in agreement with previous studies who showed that disinfection of inanimate objects may be important in controlling the spread of

enteric diseases in DCCs [22, 23]. A decreased prevalence of astrovirus was observed in DCCs that cleaned toys daily in the previous study [8]. Additionally, a decreased prevalence of *G. lamblia* was previously observed in DCCs performing extra cleaning of toys during a suspected outbreak [8], a measure that also decreased the clustered occurrence of *G. lamblia* in this study. However, cleaning toys daily did not decrease the occurrence of astrovirus clusters. Decreased clustered occurrence of norovirus and *Cryptosporidium* spp. was observed in DCCs performing extra cleaning of toys during outbreaks and in those cleaning toys daily, respectively. These findings suggest that the effectiveness of disinfection of inanimate objects might depend on the nature of enteropathogen occurrence, which can be sporadic or clustered. *G. lamblia* has been recovered from chairs and tables in DCCs while norovirus is known to persist for days on dry inanimate surfaces [24]. Cleaning vomit with chlorine-based products was previously associated with decreased prevalence of *G. lamblia* [8], and the same association was found here with clustering. This may reflect the effectiveness of chlorine in inactivating this protozoan parasite [25]. Clustering of norovirus occurred significantly less often in DCCs that cleaned child potties in a designated waste disposal station rather than in a normal sink, and a high number of attending children was associated with increased clustering of norovirus, reflecting its potential for person-to-person transmission [26]. A high number of attending children was also previously associated with increased norovirus prevalence, while cleaning child potties in a designated waste disposal station (rather than in a normal sink) was not [8]. This suggests that cleaning child potties in a designated station might prevents norovirus from occurring as clusters, but not from occurring as a whole.

In agreement with evidence provided for the value of exclusion policies for children with gastrointestinal symptoms in childcare facilities [27], we found that excluding children with GE significantly decreased the occurrence of *Cryptosporidium* spp. clusters. This policy was only associated with decreased prevalence of rotavirus in the previous study [8]. Interestingly, the latter study found that factors entailing transmission of enteropathogens via staff members, e.g. allowing them to have multiple daily duties, mixing between child groups, and entering the DCC only if/when free of gastrointestinal symptoms, were all associated with increased prevalence of one or more of the pathogens. However, none of these factors was associated with clustered occurrence of these pathogens. A possible explanation is that transmission via staff members does occur, but it does not result in significant clusters.

Generally, DCC characteristics associated with *G. lamblia* clustering found here were similar to those previously associated with its prevalence [8]. A peculiar risk factor for *G. lamblia* clustering was whether DCCs always reported outbreaks to local health authorities, indicating that DCCs are more likely to always report to the local health authority if they have (had) problems in controlling infection themselves. In the previous study [8], this DCC policy was not associated with *G. lamblia*, but only with astrovirus.

All clusters of rotavirus and astrovirus were classifiable as seasonal, and those of norovirus, *G. lamblia*, and *Cryptosporidium* spp. were all classifiable as non-seasonal. Astrovirus clusters peaked during winter months, which is in line with previous studies [8, 28]. In agreement with a recent study in The Netherlands and findings in Japan [29, 30], we found rotavirus clusters mainly during spring. Clusters of *Cryptosporidium* spp. were only observed in 2012, which is in accordance with the documented increase in *C. hominis* infections in the late summer of 2012 in patients presenting to the general practitioner (GP) in The Netherlands, UK and Germany [31]. Remarkably, clusters in DCCs occurred early in the year, starting in January, while the increase in GP patients was observed in July [31]. This suggests that the (predominantly asymptomatic) increase in clustering of *Cryptosporidium* spp. in DCCs might have been an early indication for the corresponding increase of symptomatic cases in the general population. *G. lamblia* had the highest median time span of clusters compared to the other enteropathogens, a reflection of its relatively continuous occurrence throughout the study period [32], thereby not showing seasonality. Surprisingly, no seasonality was observed for norovirus clusters. The occurrence of either outbreaks or sporadic cases of norovirus is often characterized by a strong seasonality, with the majority of them occurring during the winter [33]. A study conducted in England and Wales [34] found that norovirus outbreaks in community settings like hospitals and residential facilities were more common from November to April than during the rest of the year. However, they also found that outbreaks in settings like private homes, holiday camps, and military bases displayed no winter peak. Although the latter study only focused on outbreaks, this would provide an explanation as to why there seems to be no clear seasonal pattern in the occurrence of norovirus clusters. However, our stool samples were taken from both symptomatic and asymptomatic children. This sampling scheme might detect norovirus also when exposure is low and infection has a higher probability to evolve asymptomatically, resulting in a non-seasonal pattern. In 2012 and 2013, no clusters of norovirus were identified, which may be explained by the

way the SaTScan algorithm calculates the number of expected cases based on those occurring during the whole study period. Indeed, the relatively low prevalence of norovirus during 2012 and 2013 resulted in the absence of clusters detected in these years, with the exception of January 2012.

This study has several limitations. The questionnaires were self-administered by the DCCs. Although it is unlikely that the reported facility characteristics were not reported truthfully, it is possible that there were discrepancies between the actual and perceived behaviours towards policies in place in the DCC. Another limitation is that we could not differentiate between actual preventive measures taken against suspected outbreaks or only intentions to do so, or if this has been changed over time. This is because the data did not allow us to identify DCCs suspecting an outbreak during the study period and how they handled that. We were also unable to differentiate between enteropathogens that were introduced, but not transmitted in the DCC environment, and enteropathogens transmitted within the DCC. Yet, it seems unlikely that the introduction of an enteropathogen via one DCC attendee would lead to clustering if not further transmitted within the DCC. This is because the identified clusters were always comprised of multiple faecal samples positive for the enteropathogen in question, meaning that multiple DCC attendees would have introduced the same enteropathogen in a short time period, which is less likely to happen. Selection bias may also have occurred because faecal samples were only obtained from children while they were attending the DCCs and not from children who were absent due to illness. However, pathogen shedding can also occur before and after the symptomatic phase, which is likely to have been detected by our sampling scheme. Since none of the enteropathogens of this study were bacteria, they were unlikely to have been eradicated by antibiotic treatments and they may still have been shed after the child re-entered the DCC. Besides, antibiotic treatment in The Netherlands is prescribed in <1% of children with GE visiting the GP [5]. Although DCCs were asked to sample children at random, the possibility that some samples were not collected completely at random cannot be excluded. Finally, in the risk factor analysis, clusters were treated as present or absent at any point in time (month) independently of their size (i.e. number of samples positive for the pathogen in question). However, such data structure took into account the cluster duration, which is correlated to cluster size. Future improvements in this field should consider differentiating between clusters based on their size.

Conclusions

This study provides novel insights in the socio-demographic, facility- and policy-related DCC characteristics associated with enteropathogen clustering in DCC attendees. It also indicates that consideration of the nature of enteropathogen occurrence is important when determining targets for control, as risk factors for clustering may be different than those for enteropathogen prevalence as a whole. This study provides targets for reducing the burden of gastrointestinal morbidity in DCC-attending children, such as sandpits and paddling pools, which were the main risk factor identified for the clustered occurrence of several enteropathogens. It also highlights the importance of hygiene policies enforced in the DCC, as cleaning vomit with chlorine-based products, cleaning toys (during suspected outbreaks), and cleaning child potties in designated waste disposal stations were found to reduce the risk of experiencing enteropathogen clustering. These findings help to understand how DCC characteristics can be related to the observed clustering pattern and may enhance future interventions.

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CHAPTER 7

Different risk factors for infection with Giardia lamblia assemblages A and B in children attending day-care centres

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Published in European Journal of Clinical Microbiology & Infectious Diseases, 2016

Abstract

Background

Giardia lamblia is a major cause of diarrhoea in children, especially those attending day-care centres (DCCs). Only Giardia assemblages A and B infect humans. Given the lack of assemblage-specific epidemiological data, we aimed to identify risk factors for infection by assemblages A and B in DCC-attendees.

Methods

During 2010–2013, 5,015 faecal samples from ≤4-year-old children attending 40 DCCs participating in laboratory surveillance in the Netherlands were tested for Giardia using RT-PCR. Giardia -positive samples were typed for identification of assemblages A and B. We compared child- and DCC-level characteristics of Giardia-positive children with those of Giardia-negative children using mixed-effects logistic regression.

Results

Overall, 226 samples (4.5%) tested positive for Giardia and assemblages were determined for 138 of them: 62 (45%) were assemblage A and 76 (55%) were B. The only risk factor for assemblage A infection was attending DCCs with indoor sandpits and cats during spring/summer (odds ratio [OR] 13.5; 95%CI 1.8–101.3). For assemblage B, risk factors were attending DCCs with dedicated diaper-changing (OR 3.6; 95%CI 1.7–7.6) and laundry (OR 2.3; 95%CI 1.1–4.9) areas. Preventing sick children from attending day-care and having cloth-towels at the DCC decreased the risk of assemblage B infection (OR 0.0; 95%CI 0.0–0.5 and OR 0.3; 95%CI 0.1–0.6, respectively).

Conclusions

Risk factors for assemblages A and B infection in DCC-attending children were different, with assemblage B being mainly related to anthroponotic transmission, and assemblage A being related to zoonotic transmission. Given these differences, interventions to reduce the burden of childhood giardiasis cannot ignore those assemblage-specific preferred reservoirs and transmission routes.

Introduction

Giardia lamblia (synonyms: *Giardia duodenalis* and *Giardia intestinalis*) is one of the most common intestinal parasites of humans [1]. At present, there are eight distinct assemblages of *Giardia* (designated as A to H), of which only assemblages A and B are known to infect both humans and various animals [2], whereas the other six assemblages (C-H) are restricted to animals with narrow host ranges [3,2]. *Giardia* is the etiological agent of giardiasis, a gastrointestinal infection that is asymptomatic in the majority of persons, but may also be severe or become chronic [4], and may be harmful for the growth and development of children [5-8]. The reasons for the varying clinical manifestations remain largely unclear, but might depend on host-related factors such as the immune response and variation in the virulence and pathogenicity of *Giardia* strains [9,2,10,11]. Transmission to humans can occur through ingestion of *Giardia* cysts in food, water, or the environment contaminated with faecal material, as well as directly through the faecal-oral route in conditions of poor hygiene or where the intensity of contacts is high, such as in day-care centres (DCCs) [2].

In the Netherlands, about half of pre-school children are cared for in DCCs for an average of 2.5 days a week, and they are twice as likely to be *Giardia*-positive as their home-cared counterparts [12,13]. *Giardia* has been detected in 4.2% of DCC-attending children in the Netherlands [14], accounting for 8% of the gastroenteritis morbidity in this child population [15]. As indicated by several studies reporting epidemiological differences between assemblages A and B [16,2,1,17], potential assemblage-specific reservoirs and transmission routes may exist, the knowledge of which is still rather limited.

Although several studies have shown the importance of person-to-person transmission, travel, food, (drinking/recreational) water and personal hygiene in *Giardia* transmission [18-22], the identification of assemblage-specific risk factors in the overall population has only been addressed by two studies. The first study was conducted in 2011 in Malaysia and found infection with assemblage A to be associated with keeping pets in the household, whereas infection with assemblage B was associated with the presence of children in the household and other family members with *Giardia* infection [23]. The second study was carried out in England in 2012 and found that infection with assemblage A was associated with dog ownership, while exposures related to contact with young children and diarrhoeic people were consistently and exclusively associated with assemblage B infection [17].

These studies highlighted that preferred reservoirs and transmission routes might well be assemblage-specific. Furthermore, the role of animals in the transmission of *Giardia* to humans is still unclear despite its zoonotic potential being recognized by the World Health Organization (WHO) about 30 years ago [24]. Several studies suggested that assemblage A, which is more often detected in companion animals than assemblage B, has a greater zoonotic potential than assemblage B, but this has never been proven conclusively [2,1,16].

Given that giardiasis is a major contributor to the burden of gastroenteritis among DCC-attending children and that assemblage-specific epidemiological data are lacking for this child population, the aim of this study was to identify factors associated with infection with *Giardia* assemblages A and B in children attending DCCs.

Methods

Data collection methods

From March 2010 to March 2013, faecal samples were collected from ≤4-year-old children attending a network of 40 DCCs participating in laboratory surveillance in the Netherlands (Dutch acronym; KIzSS network), described in detail elsewhere [25,20,26]. Monthly, each DCC submitted ten faecal samples from ten randomly selected children, regardless of symptoms at the time of sampling. Faecal samples were tested for the presence of *Giardia* using an internally controlled qPCR as described previously [14]. *Giardia*-positive samples were further typed using an assemblage-specific RT-PCR protocol for assemblages A and B. Data on DCC characteristics, covering socio-demographics, facility design and policies for hygiene and disease control were obtained at the time of recruitment using a questionnaire that was self-administered by the DCC (Table 1). The occurrence of pre-defined symptoms (e.g. fever, diarrhoea and vomiting) were weekly reported by the DCC. Diarrhoea was defined as a sudden, non-chronic, onset of >3 episodes of watery stools per day. Vomiting was defined as a sudden, non-chronic, onset of >3 emetic episode per day.

Statistical analysis

Mixed-effects logistic regression models were used to examine associations between DCC characteristics (independent variables, see Table 1) and the following

dependent variables: (i) infection with *Giardia* assemblage A, (ii) infection with *Giardia* assemblage B, and (iii) infection with *Giardia* of unknown assemblage, using the *Giardia*-negative children as common comparison group. We also compared DCC characteristics, diarrhoea, and vomiting at the time of sampling of assemblage B positive with assemblage A positive children. A DCC-level random effect was included in the models to account for clustering (or non-independence) of children attending the same DCCs. We used the following independent variables as control variates: child gender and age group (≤12, 12–24, and >24 months), socioeconomic status (expressed as a normalized score from −4 to +4 based on income, employment and educational level per postcode area, with a high score denoting a low socioeconomic status) and urbanization degree (<500, 500–2,500, and >2,500 addresses/km^2) of the DCCs postal code area, season (winter, December–February; spring, March–May; summer, June–August; autumn, September–November) and calendar year (2010 to 2013). DCC characteristics with a p-value ≤0.10 were selected for inclusion in the multivariable analysis; models were built in backward stepwise fashion, with characteristics with a p-value <0.05 being retained. The effect of removing variables from the models was monitored; a change of >10% in the coefficients of the other covariates was considered as a sign of confounding, and the variables in question were retained regardless of significance. Biologically plausible interactions between independent variables were also tested. Associations were expressed as adjusted odds ratios (aORs) with corresponding 95% confidence intervals (95%CIs). Statistical analysis was performed using STATA v13.0 software (StataCorp, College Station, TX, USA).

Results

In total, 226 out of 5,015 (4.5%) samples tested were positive for *Giardia*, with a median of 3.1% among DCCS (range: 0.0–25.3%). *Giardia*-positive children had a median age of 28 months (interquartile range (IQR) 20–33 months) and 123 (54%) were male. Genotyping was successful for 146 (65%) *Giardia*-positive samples, of which 66 (45%) were of assemblage A and 80 (55%) were assemblage B, and 88 (35%) samples could not be genotyped. The median age was 28 months (IQR 20–35 months) for assemblage A-positive children and 27 months (IQR 17-32 months) for assemblage B-positive children. Thirty-five (57%) assemblage A-positive children and 38 (50%) assemblage B-positive children were male.

Table 1. Day-care centres characteristics covering socio-demographics, facility design and policies for hygiene and disease control, the Netherlands, 2010-2013 [20]

Day-care centre characteristics	**Number of day-care centres** (n=40) (%)
Hygiene policies	
Cleaning toilets less frequently than daily	34 (92)
Cleaning toys less frequently than daily	32 (89)
Cleaning vomit with paper[1]	22 (55)
Cleaning child potties in a dedicated sink[2]	12 (30)
Cleaning bed linen less frequently than daily	10 (26)
Cleaning vomit with chlorine-based products	4 (10)
Facility design	
Staff members have multiple daily duties	34 (85)
Presence of dedicated child sinks	32 (80)
Presence of sandpit outside	31 (78)
Presence of dedicated child toilets	29 (73)
Presence of laundry areas	25 (63)
Presence of nappy changing areas	17 (43)
Presence of paddling pool outside	14 (35)
Presence of cats	12 (30)
Presence of cloth towels[3]	10 (25)
Presence of paddling pool inside	3 (8)
Presence of sandpit inside	3 (8)
Control measures during suspected outbreak	
Extra cleaning of toys	30 (75)
Excluding personnel with gastroenteritis	32 (80)
Excluding children with gastroenteritis	20 (50)
Notification to local health authorities	14 (35)
Assignment of ill children to separate toilets	5 (13)
No staff member mixing among child groups	5 (13)
Demographics	
Vertical child group structure (33%)	13 (33)
Mixing of personnel among child groups (16%)	6 (16)
Number of attending children	-
Child/staff ratio	-

[1]Rather than cloth towels but without cleaner; [2]Rather than in a designated waste disposal station; [3]Rather than paper towels

Diarrhoea was reported in one (1.6%) assemblage-A-positive child and six (7.9%) assemblage-B-positive children (Fisher's exact test, p=0.130). Assemblage B was significantly associated with having diarrhoea (aOR 2.7, 95%CI 1.1–6.7) but assemblage A was not (aOR 0.6, 95%CI 0.1–4.5). Vomiting was reported in one (1.6%) assemblage-A-positive child and one (1.3%) assemblage-B-positive child (Fisher's

exact test, p=0.846). Assemblages A and B were not significantly associated with vomiting (aOR 1.1, 95%CI 0.1–9.0 and aOR 1.4, 95%CI 0.2–10.9, respectively).

In some DCCs, *Giardia* was detected for a prolonged period, which could encompass the whole study period, while in other DCCs *Giardia* was absent or rarely detected (Fig. 1). In the majority of DCCs that were positive for *Giardia* for a long period, the same assemblage was repeatedly detected. However, this could shift from one assemblage to the other at a certain time point, and infection with both assemblages A and B within the same DCC at the time of sampling was also observed.

Risk factors for assemblage A

The only DCC characteristic associated with increased odds of assemblage A infection among DCC-children was attending DCCs with indoor sandpits and cats during spring/summer (aOR 9.8, 95%CI 1.7–57) compared with DCCs with no indoor sandpits and no cats (Table 2). The association was not significant when the DCCs had indoor sandpits but had no cats (aOR 2.6, 95%CI 0.1–57). Children aged 25–36 months and those aged >37 months had at increased odds of assemblage A infection (aOR 3.9, 95%CI 1.6–9.1 and aOR 5.8, 95%CI 2.2–15, respectively), compared with children ≤12 months of age.

Risk factors for assemblage B

For infection with assemblage B, significant risk factors were attending DCCs with a dedicated diaper-changing area (aOR 3.4, 95%CI 1.6–7.0) or laundry area (aOR 2.2, 95%CI 1.1–4.8). Having cloth-towels at the DCC (aOR 0.1, 95%CI 0.0–0.5) and preventing children with gastroenteritis from entering the DCC (aOR 0.0, 95%CI 0.0–0.4) were protective factors for assemblage B infection (Table 3). Compared with children aged ≤12 months, children aged 25–36 and >37 months had increased odds of assemblage B infection (aOR 5.2, 95%CI 2.3–11.8 and aOR 4.5, 95%CI 1.6–12.2, respectively).

Risk factors for Giardia infection of unknown assemblage

Children with *Giardia* of unknown assemblage were more likely to attend DCCs that i) allowed personnel to mix among child groups (aOR 4.7, 95%CI 2.8–8.1), ii) cleaned child vomit with paper towels (but without cleaner) rather than with cloth towels (aOR 2.7, 95%CI 1.4–5.4), and iii) had indoor sandpits and cats, compared with DCCs that did not have either indoor sandpits or cats (aOR 2.4, 95%CI 1.1–5.1) (Table 2). Extra cleaning of toys during a suspected outbreak was protective for infection with *Giardia* of unknown assemblage (aOR 0.3, 95%CI 0.1–0.5).

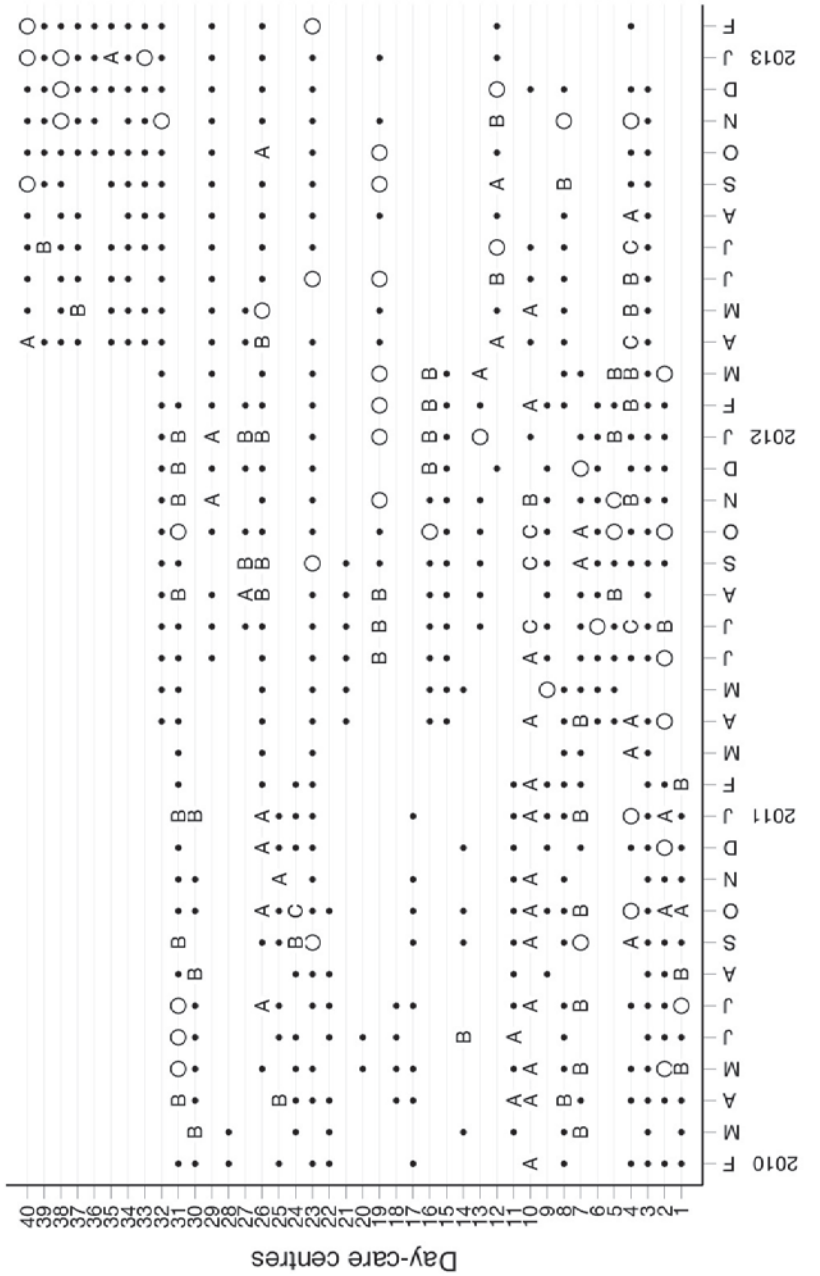

Figure 1. Detections of *G. lamblia* assemblages A and B in 40 day-care centres from February 2010 to February 2013, the Netherlands

Table 2. Adjusted odds ratios (aOR) and corresponding 95% confidence intervals (95%CI) for the association between day-care centre (DCC) characteristics and infection with *Giardia* assemblages A and B among day-care-attending children, the Netherlands, 2010-2013 (final mixed effects logistics regression model)

	***Giardia*-positive** (%)	***Giardia*-negative** (%)	**aOR (95%CI)**
Assemblage A[1]	*n* = 62	*n* = 4789	
Childs age	7 (11.3)	1188 (24.9)	Reference
≤12 months	12 (19.4)	1847 (38.6)	1.0 (0.4-2.7)
13-24 months	28 (45.2)	1332 (27.9)	3.9 (1.6-9.1)
25-36 months	14 (22.6)	356 (7.5)	5.8 (2.2-15.3)
>37 months			
Sandpits inside and cats within the DCC			
Sandpits inside and no cats	20 (32.3)	3215 (67.1)	Reference
No sandpits inside but with cats (spring and summer)	34 (54.8)	1341 (28.0)	1.7 (0.4-7.1)
Sandpits inside but no cats (spring and summer)	7 (11.3)	193 (4.0)	3.1 (0.1-68.8)
Sandpits inside and cats (spring and summer)	1 (1.6)	40 (0.8)	13.5 (1.8-101.3)
Assemblage B[2]	*n* = 76	*n* = 4789	
Childs age	7 (9.5)	1188 (24.9)	Reference
≤12 months	21 (28.4)	1847 (38.6)	2.1 (0.9-5.1)
13-24 months	46 (50.0)	1332 (27.9)	5.2 (2.3-11.8)
25-36 months	9 (12.2)	356 (7.5)	4.5 (1.6-12.2)
>37 months			
Presence of dedicated nappy-changing area	50 (65.8)	2803 (58.5)	3.6 (1.7-7.6)
Presence of dedicated laundry room	59 (77.6)	3093 (64.6)	2.3 (1.1-4.9)
Presence of cloth towels	11 (14.5)	1595 (33.3)	0.3 (0.1-0.6)
Excluding children with gastrointestinal symptoms from DCC	1 (1.3)	805 (16.8)	0.0 (0.0-0.5)
Assemblage unknown[3]	*n* = 88	*n* = 4789	
Childs age	4 (4.6)	1188 (24.9)	Reference
≤12 months	24 (27.3)	1847 (38.6)	3.4 (1.2-10.0)
13-24 months	40 (45.5)	1332 (27.9)	8.5 (3.0-24.1)
25-36 months	19 (21.6)	356 (7.5)	11.2 (3.7-33.9)
>37 months			
Urbanization degree	14 (15.9)	663 (13.8)	Reference
500-2500 addresses/km2	47 (53.4)	3628 (75.8)	1.0 (0.5-2.3)
<500 addresses/km2	27 (30.7)	498 (10.4)	2.3 (1.2-4.3)
>2500 addresses/km2			
Sandpits inside and cats within the DCC			
No sandpits inside and no cats	39 (44.3)	3192 (66.7)	Reference
No sandpits inside but with cats	27 (30.7)	1167 (24.4)	1.2 (0.7-2.2)
Sandpits inside but no cats	4 (4.6)	63 (1.3)	2.3 (0.6-8.6)
Sandpits inside and cats	18 (20.5)	367 (7.7)	2.4 (1.1-5.1)
Mixing of personnel among child groups	39 (44.3)	49 (55.7)	4.7 (2.8-8.1)
Cleaning vomit with paper[4]	70 (79.6)	3163 (66.1)	2.7 (1.4-5.4)
Extra cleaning of toys during an outbreak	53 (60.2)	3482 (72.7)	0.3 (0.1-0.5)

[1]Adjusted for urbanization degree, season and calendar year; [2]Adjusted for urbanization degree and socioeconomic status; [3]Adjusted for season and calendar year; [4]Rather than cloth towels but without cleaner

Assemblage B versus assemblage A

For infection with assemblage B compared with assemblage A, attending DCCs in rural areas (<500 addresses/km^2) was protective (aOR 0.1, 95%CI 0.0–0.5), whereas attending DCCs in highly urbanized areas (>2,500 addresses/km^2) was associated with increased risk (aOR 86.5, 95%CI 5.8–1293.9), using the DCCs in areas with 500-2,500 addresses/km^2 as reference group (Table 3). Children attending DCCs that performed extra cleaning of toys during a suspected gastroenteritis outbreak had decreased odds of infection with assemblage B, compared to that with assemblage A (aOR 0.1, 95%CI 0.0–0.3). Other risk factors for infection with assemblage B vs A were a lower socioeconomic status (aOR 2.5, 95%CI 1.3–5.0), increasing number of attending children (19–40 children, aOR 4.0, 95%CI 1.2–13.9; ≥41 children, aOR 11.6, 95%CI 2.7–49.5, with ≤18 children being the reference) and DCCs with dedicated diaper-changing areas (aOR 5.0, 95%CI 1.3–18.7).

Table 3. Adjusted odds ratios (aOR) and corresponding 95% confidence intervals (95%CI) for the association between day-care centre characteristics and infection with *Giardia* assemblage B as compared with infection with assemblage A among day-care-attending children, the Netherlands, 2010-2013 (final mixed effects logistic regression model)

	Assemblage B (%)	**Assemblage A** (%)	**aOR (95%CI)**
Assemblage B versus assemblage A[1]	N=76	N=62	Reference
Urbanization degree			0.1 (0.0-0.5)
500-2500 addresses/km2	56 (73.7)	56 (90.3)	87.5 (5.8-1330.0)
<500 addresses/km2	5 (6.6)	5 (8.1)	2.5 (1.3-4.9)
>2500 addresses/km2	15 (19.7)	1 (1.6)	
Socioeconomic status (continuous)	-	-	Reference
Number of attending children			4.0 (1.2 - 13.9)
≤18 children	19 (25.0)	31 (50.0)	11.6 (2.8-49.2)
≥19 and ≤40 children	31 (40.8)	22 (35.5)	0.1 (0.0-0.3)
≥41 children	26 (34.2)	9 (14.5)	5.0 (1.3-18.9)
Extra cleaning of toys during an outbreak	49 (64.5)	57 (91.9)	
Presence of dedicated nappy-changing area	50 (65.8)	46 (74.2)	

[1]Adjusted for age, gender, season and calendar year

Discussion

We combined epidemiological and molecular data to identify factors associated with infection with *Giardia* assemblages A and B in children attending DCCs and provided evidence which suggested that their reservoirs and transmission routes

are different. While assemblage A infections seemed to be related to zoonotic transmission, with cats being identified as a possible source of infection within the DCC, those caused by assemblage B were mainly associated with factors denoting anthroponotic transmission.

The association between infection with assemblage A and having both indoor sandpits and cats in the DCC during spring/summer suggests transmission through faecal contamination of sandpits by cats, especially because this association was not significant in DCCs that had indoor sandpits but no cats. Indiscriminate defecation of cats in sandpits is a well-known issue posing a risk also for other zoonotic infections [27,28], and is in agreement with the typical feline behaviour of preferential defecation on soft soils such as sand to bury the excreta. Our finding agrees with the Malaysian study mentioned in the introduction, as owning dogs and cats was associated with infection with assemblage A [23]. Similar findings were also reported in the English study, which reported an association between assemblage A and dog ownership [17]. Moreover, a previous study based on the data from the KIzSS network also reported an association between *Giardia* (regardless of assemblage) and the presence of indoor/outdoor sandpits in the DCC [20]. In the present study, no association was found between assemblage A and having either outdoor sandpits or cats. Possibly, this is because faecal contamination of outdoor sandpits, which occurs frequently if kept uncovered when not in use [28-30], does not entirely depend on the ownership of cats by the DCC; free-ranging cats and other animals (e.g., dogs, wildlife, etc.) that are not directly owned/cared for by the DCC can also contaminate outdoor sandpits, if/when these are accessible. In contrast, it is conceivable that contamination of indoor sandpits is likely to depend on the presence of cats that are owned/cared for by (and therefore likely to be allowed to enter into) the DCC. However, we were unable to confirm the presence of shared *Giardia* genotypes in the cats and children of the same DCCs because sampling of animals was not part of the study design. Moreover, we were only able to assess the zoonotic potential of cats, as the presence of other animals like dogs, which are known to be able to carry *Giardia*, was rare in our DCCs. Cats are known carriers of assemblage A and F, but not of assemblage B [16]. Moreover, it has been shown that assemblage A is more often detected in companion animals than assemblage B, suggesting that assemblage A has a greater zoonotic potential than assemblage B [2,1,16]. However, evidence is not conclusive

In contrast, assemblage B was mainly associated with factors mirroring hygiene standards of the DCC environment and possibly person-to-person transmission. For

instance, we found that children attending DCCs with a dedicated diaper-changing area were at increased risk for infection with assemblage B. Indeed, centralizing all diaper-changing operations might contribute to cross-infecting children having their diapers changed (often multiple times a day) in the same area, which could then serve as a 'hub' for *Giardia* [20]. This is in line with findings from England, where an association between assemblage B infection and changing diapers was found [17], as well as with previous research reporting diaper change to be a risk factor for *Giardia* infection as a whole [31,32]. The abovementioned hub hypothesis might also apply, to some extent, to areas (within the DCC) dedicated to laundry activities. Another explanation could be that care-takers getting infected when changing diapers can in turn infect other DCC-attending children. This could possibly also explain the positive association found between the presence of dedicated laundry areas and assemblage B. As DCCs do not rely on external laundry services but do it internally, DCC personnel might acquire *Giardia* by handling/washing contaminated cloths.

Similarly to assemblage A and in agreement with literature, increasing age was associated with a higher risk for infection with assemblage B [14,5]. Older children become increasingly mobile, are not yet fully toilet-trained, and hence are at higher risk for person-to-person transmission compared with young children who use diapers and are often confined to cribs.

Excluding children with gastrointestinal symptoms from the DCC was found to reduce the risk for assemblage B infection. This is supported by previous studies reporting contact with young children or with people with diarrhoea, including family members with giardiasis, to be associated with assemblage B infection [17,23]. Surprisingly, we also found that the availability of cloth (rather than paper) towels to dry hands after hand washing at the DCC was associated with decreased risk of assemblage B infection. The underlying reason is unclear. Although drying hands after hand washing has been proved to remove pathogens [33], we have no information on whether DCCs had any alternative for hand drying when no cloth towels were available. Besides, paper towels might run out and not be refilled immediately, making it difficult for personnel and children to dry their hands at any time. Whatever the reason might be, similar to the other risk factors for assemblage B infection identified here, it seems that the transmission of assemblage B is associated with the hygiene policies enforced in the DCC. Also the case–case analysis comparing children with assemblage B infection *vs.* those infected with assemblage A supports this notion. For instance, extra cleaning of toys during a suspected gastroenteritis outbreak was a specific protective factor for assemblage B

infection as compared with assemblage A, suggesting that hygiene practices might be more successful in preventing assemblage B, rather than assemblage A infections.

The risk for assemblage B infection also increased with increasing number of children in the DCC, further supporting the role of anthroponotic transmission (even if mediated by the environment) in the epidemiology of assemblage B. Furthermore, while assemblage A infections occurred significantly more often in children attending DCCs in rural areas, those caused by assemblage B were more likely to occur in children attending DCCs in highly urbanized (and therefore more densely populated) areas. This could reflect more opportunities for person-to-person transmission of assemblage B when surroundings are relatively more (over) crowded, as well as more opportunities for zoonotic transmission of assemblage A in less densely populated areas where animals (livestock and wildlife) are generally more likely to dwell. Another possible explanation for assemblage B being mainly associated with factors suggesting anthroponotic transmission could be that assemblage B, in contrast to A, was associated with having gastroenteritis at the time of sampling. Children with diarrhoea might be more likely to contaminate the environment compared with those without diarrhoea due to increased stool frequency. However, it is unlikely that the above fully explains the association, since the majority of children positive for assemblage B did not have diarrhoea. Although several studies reported assemblage-related clinical outcomes, their results are inconsistent [34-37].

Risk factors for infection with *Giardia* of which the assemblage could not be determined were a mixture of characteristics suggesting both anthroponotic and zoonotic transmission. This indicates that these *Giardia*-positive samples probably comprised both assemblages A and B.

This study has several limitations. First, DCCs were asked to sample children at random, but some convenient sampling might also have occurred. Indeed, it might have been easier to sample children still in diapers compared with those with some toilet training. However, children attending DCCs often use potties instead of toilets, which are as easy to sample as diapers. In any case, differences among DCCs with respect to sampling preferences were accounted for by taking into account the effect of clustering in the analysis. Second, selection bias of children with loose stools might have occurred when present in the DCC due to increased stooling frequency, providing more sampling opportunities. However, the effect on the prevalence estimates is considered to be limited, since, in contrast, children with loose stools might be kept home due to illness. Third, *Giardia* is known to be shed intermittently;

thus, our sampling scheme might have underestimated its true morbidity, as infected children might not have shed cysts at the time of sampling. Fourth, questionnaires were self-administered, which possibly led to some discrepancies between perceived and actual behaviour with respect to, for instance, hygiene practices, given that the questionnaire was filled in by the head of the DCC instead of by actual nursing staff. However, it is highly unlikely that information on DCC facilities, such as the presence of sandpits, were not reported truthfully. Finally, no information was available on potential risk exposures outside the DCC, nor household characteristics of the sampled children, which could have influenced the associations found here. For example, parents who own pets such as cats might be more inclined to enrol their child in DCCs that also have pets.

Our findings indicate that epidemiologically relevant differences in preferred reservoirs and transmission routes do exist between *Giardia* assemblages A and B infecting children that attend DCCs. While assemblage A seemed to be possibly related to zoonotic transmission, with contamination of indoor sandpits by cats, assemblage B was mainly associated with factors related to hygiene practices within the DCC, denoting mainly anthroponotic transmission. The existence of two distinct epidemiological profiles for assemblages A and B suggest that assemblage-specific interventions are needed to reduce DCC-associated gastrointestinal morbidity, as their outcome with regard to giardiasis may largely depend on the circulating assemblage.

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CHAPTER 8

Marked decrease in rotavirus detections among preschool children unvaccinated for rotavirus in the Netherlands, 2014

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Published in The Pediatric Infectious Disease Journal, 2016

Abstract

Rotavirus detection rates among preschool children sampled irrespective of symptoms during the rotavirus season (January-April) in the Netherlands were significantly lower in 2014 (0.6%) than in 2010 (11.2%), 2011 (6.9%), 2012 (6.8%), and 2013 (6.7%). This supports previous observations of a genuine drop in rotavirus circulation (without rotavirus vaccination) rather than a milder disease course.

Introduction

Rotavirus is the leading cause of diarrheal disease in children of preschool age. Between August 2013 and July 2014, the number of notified cases of rotavirus infection detected by passive virologic surveillance in the Netherlands dropped by 58% compared with the same period of the previous years [1]. This drop comprised mainly symptomatic patients because this surveillance system gathers data on virus detections from diagnostic laboratories joining the Dutch Working Group of Clinical Virology (NWKV), serving primary care units, hospitals, and long-term facilities. Additionally, the weekly consultation rate for gastroenteritis (GE) in <5-year-old children in the Netherlands dropped from 152/100,000 in 2006 to 2013 to 97/100,000 in 2013 to2014 (–36%). In 2014, a concurrent decrease in rotavirus notifications was observed in the geographically close countries UK [2] and Germany [3], where rotavirus vaccination is part of their national immunization programs. However, rotavirus vaccination is not part of the Dutch immunization program, and there have been no changes in laboratory methods and reporting procedures that may explain the observed drop. We hypothesized that this drop might be because of a milder course of the disease, resulting in less rotavirus-associated hospitalizations and visits to the general practitioner requiring stool testing and case notification.

Using passive surveillance-independent data, we corroborated whether the observed decrease in rotavirus infections in the Netherlands in 2014 was caused by a genuine drop in rotavirus circulation among children less than 5 years of age.

Materials and methods

Data from 2 comparable population-based studies were used. Data from the first study consisted of rotavirus detections in stool specimens of 5718 <5-year-old children attending a network of 44 Dutch day-care centers (DCCs) participating in laboratory investigation of enteropathogens during February 2010 to February 2013, the "KIzSS" study [4]. Monthly, each DCC's nursery staff submitted 10 stool specimens from 10 randomly selected children regardless of their symptoms. Data from the second study, the "Family&Health" study [5], consisted of rotavirus detections in stool specimens of 927 <5-year-old children from a sample of 8768 Dutch households with preschool children during April 2013 to September 2014. A sample of 2000 preschool children living in different households was drawn monthly

from Dutch population registries, and their parents were invited to submit a stool specimen of that child and one parent regardless of symptoms. Stool specimens from both studies were examined for the presence of rotavirus using an internally controlled multiplex reverse transcriptase polymerase chain reaction.

Rotavirus detections in January to April, the usual rotavirus season in the Netherlands [1], were compared between years (2010–2014, with 2014 being the reference). Analysis was performed using logistic regression models incorporating cluster-robust standard errors to account for clustering of children at the DCC level; estimates were expressed as age- (categorized as ≤6, 7–12, 13–36 and 37–48 months old), and gender-adjusted mean rotavirus detection rates with corresponding 95% confidence intervals (95%CI). Statistical analysis was performed with STATA 13, and statistical significance was set to $P < 0.05$.

Hospitalization data from the National Disease Registry, collecting data on hospital discharges from all Dutch hospitals, were analyzed for the International Classification of Disease codes for GE suspected for rotavirus (ICD9 codes 86–93 and 5589) in <5-year-old during 2010 to 2014. GE diagnoses in 2014 were compared with those in 2010 to 2013 using negative binominal regression models. Additionally, previously published data [1] on general practitioner consultations for all-cause GE in <5-year-olds and detected cases of rotavirus infection determined by passive virologic surveillance were updated to July 2015.

Results

From March 2010 to September 2014, 6542 stool specimens were tested for rotavirus. The median age of these children was 21 months (interquartile range: 13–29 months); 3339 (51.8%) children were male, and 6133 (93.8%) attended a DCC. In total, 195 (3.0%) children were rotavirus-positive and had a median age of 14 months (interquartile range: 8–21 months). Ninety-seven children (50.3%) were male.

Most (71.3%) rotavirus detections occurred during the usual rotavirus season (January–April). The adjusted rotavirus detection rate in January to April 2014 (0.6%, 95%CI 0.0%–1.8%) was significantly lower than that of the same period in 2010 (11.2%, 95%CI: 5.6%–16.9%; $P < 0.001$), 2011 (6.9%, 95%CI: 1.8%–12.1%; $P = 0.009$), 2012 (6.8%, 95%CI: 3.7%–10.0%; $P < 0.001$) and 2013 (6.7%, 95%CI: 3.6%–9.7%; $P < 0.001$).

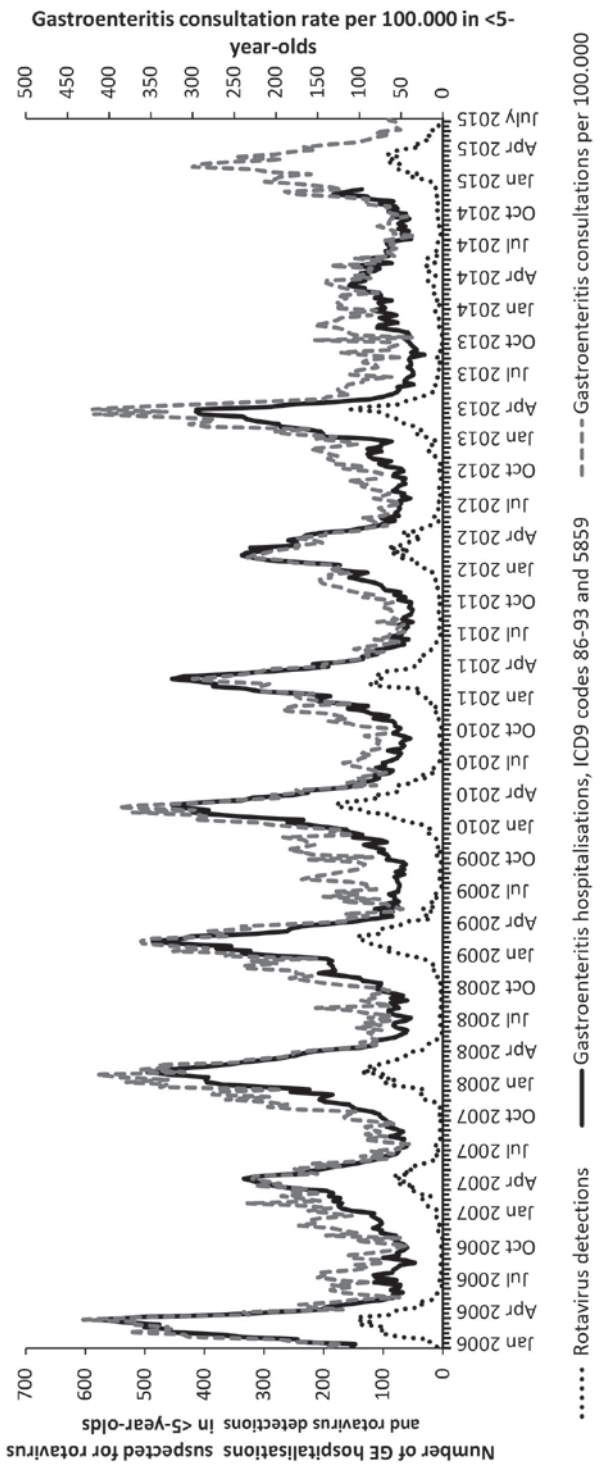

Figure 1. Weekly rotavirus detections, general practice GE consultation rates in <5-year-old children, and hospitalizations for GE suspected for rotavirus (ICD-codes 86-93 and 5859) among <5-year-old children during January 2006 – July 2015, the Netherlands.

Compared with ≤6-month-old children, those aged 13–36 and ≥37 months of age had a significantly decreased odds for rotavirus infection (odds ratio 0.3, 95%CI: 0.2–0.4; P <0.001 and odds ratio 0.2, 95%CI: 0.0–0.5; P =0.001, respectively).

A total of 71,512 children <5 years old were hospitalized for GE during 2010–2014 suspected for rotavirus. In 2014, children were significantly less hospitalized for GE suspected for rotavirus, as compared with 2010 (incidence-rate ratio [IRR] 1.6, 95%CI: 1.3–2.0), 2011 (IRR 1.5, 95%CI: 1.2–1.9), 2012 (IRR 1.4, 95%CI: 1.1–1.7) and 2013 (IRR 1.3, 95%CI: 1.0–1.6). Rotavirus detections, GE consultations and hospitalizations for GE suspected for rotavirus had very similar temporal patterns and were all much lower in 2014 than in earlier years (Fig. 1).

Discussion

We experienced a significant decrease in rotavirus detections in 2014 during the usual rotavirus season among <5-year-old children sampled at random, regardless of symptoms and independently of the 3 passive surveillance systems operating in the Netherlands. This provides evidence that the concurrent decrease in the laboratory surveillance of rotavirus in patients [1] was a result of a genuine drop in rotavirus circulation in the target child population, and unlikely the result of a less severe course of the disease. This finding was supported by the decrease in children hospitalized for GE, to which rotavirus is one of the major contributors [6].

The use of passive surveillance-independent data to corroborate the 2014 decline in rotavirus infection among preschool children is a strong point of this study. Yet it also represents a limitation because the KIzSS study (2010–2013) focused on DCC attendees, who are at increased risk for gastrointestinal infections in general [7], whereas the Family&Health study (2013–2014) included both DCC-attending and home-cared children. However, virtually all children acquire rotavirus infection before the age of 5 years [8], and differences in rotavirus detection in 2014 between DCC-attending and home-cared children were found to be insignificant (P =0.255). Although the nursery staff was asked to collect fecal samples at random, convenience sampling might have occurred because some children might have been more likely to be sampled than others. For instance, we speculated that sampling of children still in diapers would be easier than that of toilet-trained children, although children in DCCs rarely defecate directly into the toilet, but rather use potties, which are easy to sample as well. Although DCCs were allowed to decide on their randomization

charts, differences between DCCs were then accounted for by a full analysis of clustered data.

It has been suggested that the exceptionally low rotavirus infection incidence in 2014 might result in a hyperendemic rotavirus season in the coming year(s) [1]. Interestingly, the 2015 rotavirus season followed the usual (i.e. pre-2014) pattern in the Netherlands (Fig. 1). It is therefore unlikely that the implementation of rotavirus vaccination in the United Kingdom and Germany had contributed to the 2014 drop in the Netherlands because rotavirus vaccination policy in these countries has not been changed in 2015. Alternative causes for the 2014 drop might therefore be the interplay of an unusually mild 2013/2014 winter [9], a high rotavirus incidence in previous years, and a low birth rate [10]. These might have reduced the occurrence of the 2014 rotavirus season given the low number of infants entering the susceptible population in combination with the mild winter temperatures, resulting in such a reduced risk of transmission to prevent the usual rotavirus season from occurring [10]. To better understand the epidemiology of rotavirus, continuous monitoring and ad-hoc studies are warranted in the future.

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CHAPTER 9

Biennial pattern of rotavirus gastroenteritis in the Netherlands and a shifting age distribution following a low rotavirus season, 2010-2016

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Published in The Pediatric Infectious Disease Journal, 2018

Abstract

A hyperendemic rotavirus season was expected after a low-endemic 2014 season in the Netherlands. Rotavirus detections were however similar in 2015 and lower in 2016 compared with 2010–2013. Gastroenteritis consultation rates were also similar in 2015, but the age-distribution shifted to older children because of an accumulation of noninfected children. Results indicate a possible shift to a biennial rotavirus pattern.

Introduction

An unexpected 58% decrease in rotavirus (RV) detections was observed in virologic laboratory surveillance in the Netherlands during the 2014 RV epidemiologic year (August 2013 to July 2014), while RV vaccination is not included in the National Immunization Programme. Similarly, gastroenteritis (GE) consultations of children younger than 5 years of age at general practitioners (GPs) decreased by 36% in 2014 compared with previous years[1]. Moreover, the seasonal annual peak shifted from March to May. A concurrent decrease in RV incidence was reported in preschool children in day-care sampled at random and regardless of symptoms[2], providing evidence that the lower number of RV detections was caused by a genuine drop in RV circulation rather than altered pathogenicity of the virus. Simultaneously, a decrease occurred in those younger than 5 years of age with RV-suspected GE hospitalizations[2].

The drop in RV activity in 2014 remains largely unexplained. A hyperendemic RV season was expected in the following year(s) because of an increased number of susceptible children unaffected during the low-endemic 2014 RV season[1]. However, the 2015 RV season followed the usual pattern, and in 2016, RV activity was low again[2, 3]. We hypothesized that in 2015, despite the usual RV pattern, a possible shift may have occurred toward older children because of an accumulation of susceptible children who had not been infected during the preceding low-endemic year.

Therefore, the aims of this study were (1) to quantify the 2014, 2015 and 2016 RV seasons in the Netherlands and (2) to assess a possible shift in age-specific RV incidence rates in 2015 after the low-endemic 2014 season.

Material and methods

We used anonymized weekly data of RV laboratory detections from August 2009 to July 2016, reported by the Working Group for Clinical Virology: a sentinel network of laboratories serving primary and secondary care. Second, we used weekly all-cause GE consultation rates in children younger than 5 years of age (International Classification of Primary Care, code D73) from 2010 to 2015. These data were collected by routine electronic health record extractions from GPs participating in the primary care database of the Netherlands Institute for Health Services Research (NIVEL) (~7% national coverage)[4]. Weekly rates were calculated as the number

of patients with 1 or more records for GE consultations, using the total number of patients enlisted in the practice as denominator (weekly prevalence rate).

Weekly number of RV laboratory detections in the 2014, 2015- and 2016 RV epidemiologic years (August to July) was compared with those in 2010–2013. We calculated incidence rate ratios (IRR) with corresponding 95% confidence intervals (CIs) using negative binominal regression models.

The weekly GE consultation rate in those 5 years of age and younger in the 2014 and 2015 RV season was compared with the same time period in 2010–2013. Here, we defined a season as the period from January to June to minimize distortion by other GE-causing pathogens. We calculated IRRs using negative binomial regression models, with the weekly number of children younger than 5 years of age enlisted in GPs as offset variable. The same analyses were performed stratified by age (1-, 2-, 3- and 4-year-olds). Because of the incomplete registration of 0-year-olds during the study period, age-stratified analyses were not performed for this age group. Statistical analyses were performed with STATA version 14.2 (StataCorp 2015, College Station, TX), and statistical significance was set to $P < 0.05$.

Results

Between August 2009 and June 2016, the mean annual number of RV laboratory detections during the RV epidemiologic years was 1306 (range, 551–2227) and the mean annual number of GE consultations in children younger than 5 years of age was 4182 during the RV seasons (range, 2991–5017). Both show similar seasonal patterns with annual peaks in March and the beginning of April (Fig. 1). Only in 2014 and 2016 (laboratory detections), the peak has shifted to May.

The weekly number of RV laboratory detections in the 2015 RV epidemiologic year was similar (mean, 27.0; IRR, 0.86; 95% CI: 0.62–1.19) to those in 2010–2013 (mean, 31.3; range, 1–180). However, the weekly number of RV detections in 2014 and 2016 was significantly lower (mean, 10.6; IRR, 0.34; 95% CI: 0.25–0.47 and mean, 12.7; IRR, 0.41; 95% CI: 0.29–0.56, respectively) compared with 2010–2013.

The weekly GE consultation rate in children younger than 5 years of age in the 2014 RV season was significantly lower (1.42/1000 population; IRR, 0.65; 95% CI: 0.54–0.78) compared with 2010–2013 (range, 0.69–4.73/1000 population; mean, 2.18/1000 population). During the 2015 RV season, it did not differ from 2010 to 2013 (2.03/1000 population; IRR, 0.93; 95% CI: 0.77–1.13).

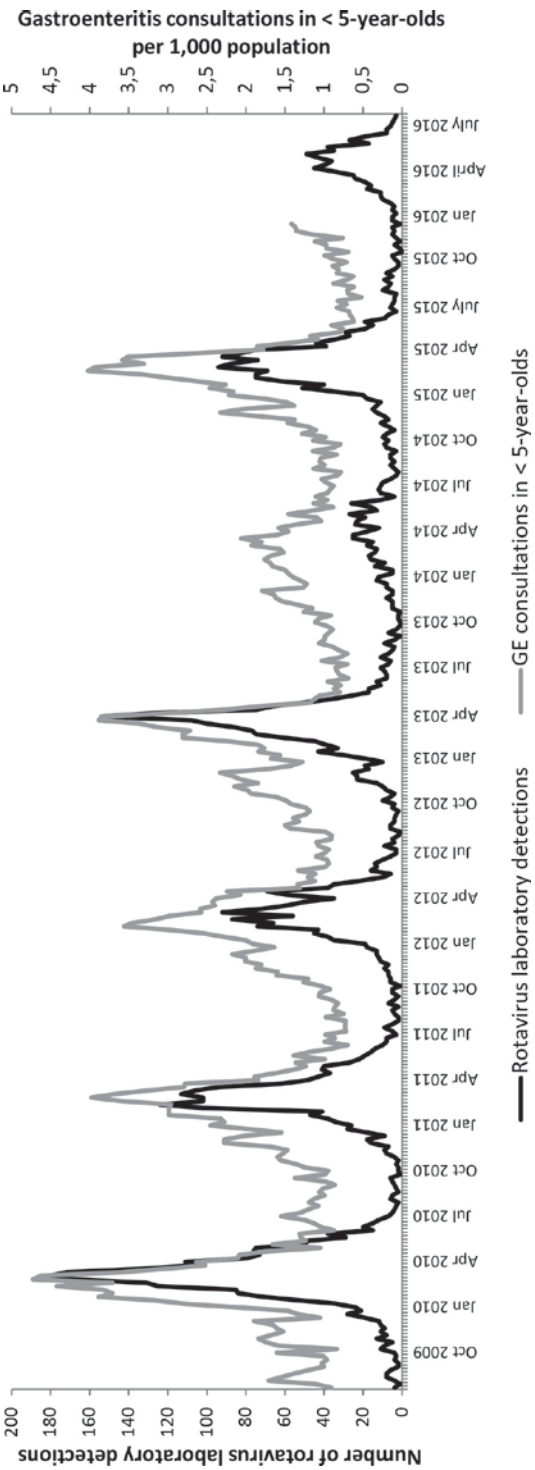

Figure 1. Weekly rotavirus laboratory detections and weekly all-cause gastroenteritis (GE) consultation rates in children younger than 5 years per 1,000 population, the Netherlands, August 2009 to July 2016.

The GE consultation rate during the 2014 RV season was significantly lower in 1-, 2- and 3-year-olds (IRR, 0.59; 95% CI: 0.49–0.72; IRR, 0.67; 95% CI: 0.55–0.82 and IRR, 0.67; 95% CI: 0.54–0.83, respectively) compared with 2010–2013. A non-significant decrease occurred in 4-year-olds (IRR, 0.85; 95%CI: 0.68–1.05). During the 2015 RV season, the GE consultation rate in 1- and 4-year-olds was similar to the rate in 2010–2013 (IRR, 0.9; 95% CI: 0.72–1.11 and IRR, 1.07; 95% CI: 0.85–1.35, respectively). However, it was significantly higher in 2- and 3-year-olds (IRR, 1.66; 95% CI: 1.34–2.07 and IRR, 1.52; 95% CI: 1.21–1.90, respectively).

Discussion

We observed in 2016 like in 2014 a significantly reduced RV activity, while the 2015 RV year was as usual. Observations were consistent between the 2 surveillance sources. Furthermore, the age-distribution of GE consultations during the 2015 RV season shifted after the low-endemic year 2014, affecting relatively more 2-and 3-year-olds.

The results in this study suggest a shift in the Netherlands from an annual to a biennial pattern. Preliminary observations of RV laboratory detections for 2017 show a continuing pattern. To our knowledge, a biennial RV pattern has not been observed before in a country without RV vaccination. A shift from annual to biennial seasons has been predicted after implementation of mass vaccination in mathematical transmission models[5] and has been reported in postvaccination years in the United States and Belgium, countries with moderate to high vaccination coverage[6, 7]. The slower accumulation of unvaccinated susceptible children that is required to set off the seasonal epidemic results in stronger RV seasons every other year, rather than every year. However, other vaccinating countries with high vaccination coverage have not reported a biennial pattern[8]. Belgium, England, Wales and Germany also observed a delay in the typical seasonal peak of RV in postvaccination years as we observed in the low-endemic 2014 and 2016 RV years, while the start of the RV seasons were similar[6]. We speculate that the pattern observed in the Netherlands might be a herd effect from vaccinating surrounding countries (Belgium, West Germany and the United Kingdom) with high RV vaccination coverages. However, to our knowledge, this phenomenon has not been described before and the mechanisms that could explain this biennial pattern in the Netherlands have yet to be revealed.

In the Netherlands, G1P[8] was the most prevalent genotype until 2011. Since then, the contribution of G1P[8] has gradually decreased, while other strains such

as G9P[8] and G4P[8] have become more prevalent[3, 9]. Both in low-endemic years 2014 and 2016, G9P[8] was the dominant strain. Although some studies indicate that severity of disease is associated with RV genotype, other studies were unable to confirm this[10, 11]. A day care study in the Netherlands showed that symptomatic and asymptomatic RV infections were reduced in 2014[2], rendering reduced pathogenicity an unlikely explanation. Recent studies have provided new insights into human genetic susceptibility to various RV strains. A sub-domain (VP8) of the human RV VP4 (P-genotype) surface protein interacts with components of the human to attach to intestinal cells [12]. Genetic polymorphisms in genes encoding histo-blood group antigens mediate host susceptibility to different RV P-genotypes, and the expression differs between ethnic populations[13]. While the dominant P-genotypes circulating in the Netherlands have been relatively stable over the years, G-genotypes have differed to a much larger extent. Both in low-endemic years 2014 and 2016, G9P[8] was the dominant strain. Yet, variations in host susceptibility to different G-genotypes have not been described so far but form an interesting hypothesis given the observed shift in G-strains and concurrent decline in RV disease in the Dutch population. In addition, we cannot confirm if the observed changes in dominant circulating genotypes are natural, or the result of vaccination in surrounding countries.

A plausible explanation for the significantly increased GE consultation rates in 2-and 3-years-olds in 2015 compared with 2010–2013 is that the preceding low-endemic 2014 RV season resulted in an accumulation of noninfected 2- and 3-year-old susceptible children in the 2015 season.

This study has some limitations. First, the laboratory data do not include information on the total number of RV tests performed. However, we believe that this has only minor impact because there were no changes in diagnostic guidelines or reimbursements policies during the study period. Second, data on GE consultations in 2016 were not yet available, and because of incomplete registration, we were unable to include 0-year-olds in age-stratified analyses. Third, distortion from other circulating enteric pathogen is possible, which was minimized by restricting our analyses of GE consultations to months of RV peak activity.

Whether the biennial pattern in the Netherlands could be driven by RV vaccination in neighboring countries, or the presence of other causes remains to be determined. A shift toward older children was observed in the year after a low-endemic RV season. Close monitoring of RV epidemiology is warranted to identify new or changing trends.

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CHAPTER 10

General discussion

Research presented in this thesis improves our understanding of the epidemiology of GE in preschool children, as well as their role in the household and societal burden of GE in the Netherlands. This contributes to fill several knowledge gaps that have arisen from previous research, but that have remained unanswered thus far. For each of the four sections in this thesis, it is discussed here below what they add to the current knowledge, as well as the public health implications of the findings.

Section I: Gastroenteritis burden in the general population and the role of preschool children

What is already known

In high-income countries like the Netherlands, GE is usually mild and self-limiting. However, it may have a severe course in vulnerable populations, such as young children, elderly, and immunocompromised persons. Despite the low mortality rate, the societal burden of GE is substantial due to the high incidence of the disease and associated productivity losses of the ill persons and/or their caregivers. In Europe, the incidence estimates of GE vary from 0.3 to 1.4 episodes/person-year, depending on the country (1-4). GE incidence was estimated at 0.9 GE episodes/person-year in Poland in 2009–2010, 1.0 episodes/person-year in Germany in 2008–2009, 1.1 episodes/person-year in Italy in 2008–2009, and 1.4 episodes/person-year in Denmark in 2009 (1-4). In France, the estimated incidence was much lower, with 0.3 episodes per person-year in 2009–2010 (5). Although population-based studies from other countries used slightly difference case definitions for GE, estimates were similar; 1.2 episodes/person-year in Norway in 1999–2000, 1.4 episodes/person-year in the United States of America in 1996–1997, and 1.2 episodes/person-year in Canada in 2005–2006 (6). In the Netherlands, GE incidence was estimated at 1.0 episodes/person-year in 2009, of which 1 in 13 cases visited the GP and 1 in 150 cases was hospitalized (7).

Although incidence rates vary between countries, they almost all report the highest incidence in children younger than 5 years of age (1-3, 5-10). In the Netherlands, the incidence of GE in children below 5 years was estimated at 2.9 episodes/person-year in 2009, which was three-fold higher than the overall community incidence (7, 11). Moreover, an increased risk for GE was observed in children that attend DCCs compared with children that are home-cared (11). Transmission from children to their parents is estimated to occur in 20% to 40% of GE episodes in children (12, 13).

In the Netherlands, the total societal costs of GE were estimated at €345 million in 2003, corresponding to €77 per GE episode (14). These costs increased to €611–695 million in 2009, which is between €133 and €155 on average per episode of GE (15). The higher costs compared with 2003 were mainly attributable to an increase in healthcare costs.

What this thesis (Chapters 2 and 3) adds

Section I (Chapters 2 and 3) provided updated estimates of the burden of GE in the Dutch community. In addition to previous research, we considered specifically the role of preschool children in the household burden of GE. Moreover, while previous research focused mostly on children attending DCCs or those needing medical attention (e.g. hospitalized children), we characterized infection risks for GE within families that have young children independently of DCC attendance or severity of GE episodes. Furthermore, we provided an update of the economic burden of community GE, comparing also the costs of GE in households with and without children, which had not been done before in the Netherlands.

In Chapter 2, the incidence of GE in all age groups of the Netherlands' general population was estimated at 0.8 episodes/person-year during 2014–2016, corresponding to 13.2 million episodes of GE each year. This is slightly lower than the previous estimate of 1.0 episodes/person-year in 2009–2010 (7). The incidence was highest in children younger than 5 years (2.0 episodes/person-year), followed by persons aged 18–24 years (1.5 episodes/person-year). The incidence then declined with increasing age to 0.5 episodes/person-year in persons ≥65 years. One in 16 GE cases visited the GP, which is in line with findings from the previous community-based study on GE in 2009 (7). The number of hospitalizations, however, was higher, with 1 in 60 GE cases requiring hospitalization compared with 1 in 150 previously estimated, but both estimates were based on small numbers. An increased risk for GE was observed among adults aged 18–44 years living in a household with children, specifically children below 5 years of age, compared with adults living without children in the household. This was not found among adults aged 45–64 years, possibly indicating increased immunological maturity of parents due to repeated exposure to enteropathogens, although the number of adults in thig age group with young children in the household was relatively low. Chapter 3 supported these findings, as the risk for GE significantly decreased with an increasing age of the parent, independently of the children's age. Intuitively, family members had more GE with an increasing number of children in the household, and the risk for GE

in parents increased with an increasing number of children attending a DCC. The risk for GE was also higher in females and in families that displayed poor food handling practices. Furthermore, DCC attendance increased GE risk in children below 5 years of age, but not beyond, probably due to immunological maturation. This is concurrent with findings from a more recent Dutch cohort study in children until age 6 years, where DCC attendance was found to increase GE burden in the first year of DCC attendance but was found to be protective afterwards as compared to home-cared children (16). Moreover, in a Danish study, attending DCCs was associated with a higher risk for hospitalization until one year of attendance (17). However, in children that started attending the DCC before the age of one, the risk of being hospitalized was lower during preschool years, but did not last in elementary school years. The use of gastric antacids, particularly proton-pump inhibitors, was associated with increased risk for GE in preschool children and in adults, likely due to increased susceptibility to infection with bacterial agents resulting from decreased bactericidal effect of gastric acidity (7, 18). Interestingly, we found that children with chronic respiratory diseases, specifically asthma, had GE more often, for which the specific underlying mechanism is unknown, although speculations can be made regarding general frailty and/or use of specific medicines that may increase GE risk in these children.

The total costs of GE in 2017 amounted to €945 million in the Netherlands, which corresponds to €191 per GE episode and €55 per inhabitant (Chapter 2). The average costs per GE episode were €126 in children 0–4 years, €61 in the age group 5–17 years, €82 in the age group 18–24 years, €182 in the age group 25–44 years, €261 in the age group 45–64 years, and €475 in persons ≥65 years. The main determinants of costs were productivity losses and hospitalization, the latter mainly in persons aged ≥65 years.

Section II: Prevalence, risk factors, clinical relevance and household transmission of enteropathogens in preschool children

What is already known

Many different bacteria, viruses, and protozoa may cause GE, with the most important causes being rotavirus and norovirus in children below 5 years in high-income countries (19-21). Most research on the etiology of GE focusses on children presenting at the GP or children admitted to hospital. However, these generally represent the more severe cases requiring medical attention, and because some

enteropathogens (i.e. potential causative agents of GE) are more likely to cause severe disease than others, they are unlikely to reflect the etiology of GE at community-level.

Infection with an enteropathogen does not necessarily lead to illness. For example, previous research found that asymptomatic carriage was very common among DCC-attending children in the Netherlands (22). Out of 16 enteropathogenic bacteria, viruses, and protozoa, only norovirus and rotavirus were significantly associated with GE, indicating that enteropathogen detection in a stool sample might often not be clinically relevant. The literature is conflicting on whether children with mixed infections are at increased risk for (severe) GE, and they mostly focus on children with GE presenting at the hospital emergency department or children that are hospitalized (23-27). To understand the clinical relevance of enteropathogen detection in stool samples, studies that are not driven by symptomatology are essential but are scarce in literature.

Taking effective preventive measures to reduce the incidence of GE requires insight in the epidemiology of GE. Enteropathogens have many different modes of transmission and may spread between persons, be acquired from ingested food or water or from environmental sources, or from exposure to animals. The relative contribution of enteropathogens to GE might change over time, as was for example observed for *Salmonella* due to Europe-wide control strategies in the animal production (28). Data demonstrating changes in the relative importance of viral, bacterial, and protozoal agents therefore need continuous updates to monitor these changes, as well as the relevance of risk factors, which may also change over time. The last population-based study on the etiology of GE was conducted two decades ago in 1998–1999 (29).

What this thesis (Chapters 4 and 5) adds

Section II (Chapters 4 and 5) of this thesis provided an update on the etiology of GE in the Dutch general population, specifically in children below 5 years of age and their parents. We also described the clinical relevance of the detection of different enteropathogen in stool samples of children, as well as their parents, and identified risk factors for infection. Furthermore, we showed that children with multiple enteropathogen co-infections are not necessarily at increased risk for GE as compared with children infected with only one enteropathogen.

In Chapter 4, a substantial overlap was found in pathogens in stools of children and their parents, suggesting extensive transmission within households, particularly

viruses. Viruses predominated during winter, bacteria in summertime, and protozoa had a less pronounced seasonal pattern. Comparing our findings on the etiology of GE with those from the population-based study in 1998–1999 was hampered by the fact that different diagnostic methods have been used for some of the enteropathogens (30). However, for sapovirus, both studies used polymerase chain reaction (PCR), and we observed a drop from 8% in the previous study to 3% in our study. Moreover, while we used a more sensitive laboratory test for adenovirus, we found only 0.1% of children positive for adenovirus type 41, compared with 5% for adenovirus type 40/41 previously. Although we only tested for adenovirus type 40, this is unlikely to explain the difference because adenovirus type 40 is much less detected than type 41 in the Netherlands (31, 32). We also used a more sensitive laboratory test for rotavirus than previously, but found only 2% of children positive for rotavirus compared with 10% previously. This is most likely because our study was performed during 2014, which had an exceptionally low rotavirus activity (see Chapter 9). We found that most enteropathogens were detected just as much in children and parents with GE as in those that did not have GE. In children, norovirus GII, astrovirus, and adenovirus type 41 were significantly more often detected in those with GE than those without GE, indicating the highest clinical relevance for viruses. In adults, norovirus GII, sapovirus, and *Cryptosporodium* were significantly associated with GE. Chapter 5 described that co-infections of viruses, bacteria, and protozoa were detected in about half of the children below 5 years of age, but were not associated with an increased risk for GE. Several enteropathogens were found to co-occur more often than expected by chance, which could indicate biological interactions between them, for which the body of literature is currently limited, but would require further testing in e.g. experimental settings (33). Interestingly, we found that children with a food allergy more often had co-infections.

Surprisingly, enteroaggregative *Escherichia coli* (EAEC) was significantly associated with exposure to horses, while humans are the only recognized reservoir. This triggered further research into horses as AEAC reservoirs. However, randomly selected faecal samples of 70 horses in an equine hospital in the Netherlands all tested negative for EAEC (34). The previously reported zoonotic potential of *Giardia* was supported by our finding that *Giardia* was observed more in children and adults living in households with a cat (Chapter 4) and in children attending DCCs that owned a cat (Chapter 7) (35).

Section III: Clustering of enteropathogens in day-care centers and the potential for zoonotic transmission

What is already known

Almost half of preschool children in the Netherlands attend a DCC for an average of two days a week (36). DCCs are especially prone to pathogen spread because it is a confined space crowded with children that have imperfect hygiene behaviours and an immature immune system (37, 38). Therefore, children attending a DCC are at increased risk for GE (11, 22). Previous research found that the main contributors to GE morbidity in DCCs in the Netherlands were rotavirus, norovirus, astrovirus, *Giardia,* and *Cryptosporidium*, accounting altogether for 39% of the morbidity (39). Several socio-demographic, facility- and policy-related DCC characteristics have been associated with these enteropathogens, as well as with GE as syndrome (40). However, risk factors might be different for sporadic cases and for cases that cluster in time (e.g. prolonged outbreak or long-term persistence). Space-time scan statistics have proved useful in identifying clusters of pathogens in community care services, such as hospitals, and may help identifying clustering of enteropathogens in the DCC, as well as the risk factors involved (41-43). For *Giardia*, previous research has suggested that risk factors may even be different between different assemblages (44-46).

What this thesis (Chapters 6 and 7) adds

Section II (Chapters 6 and 7) described risk factors that were associated with temporal clustering of the major enteropathogens, i.e. rotavirus, norovirus, astrovirus, *Giardia*, and *Cryptosporidium*, in DCCs, as well as assemblage-specific risk factors for *Giardia*. The findings provide insights into the transmission dynamics and targets for control in the DCC.

In Chapter 6, we found that clusters of these enteropathogens had different temporal patterns, with time spans varying from one month up to a year. For rotavirus and astrovirus, recurring seasonal clusters were observed, while those of norovirus, *Giardia* and C*ryptosporidium* occurred throughout the year in DCCs without seasonal pattern. Although norovirus usually has a strong seasonal peak, the absence of a winter peak was also observed in private homes, holiday camps, and military bases in England and Wales (47, 48). Clusters were observed more often in DCCs that had sandpits, which could be due to contamination of sandpits with faecal coliforms or vomit, as reported previously (49, 50). Hygiene-related

factors were associated with decreased clustering of some of the pathogens, such as extra cleaning of toys during a suspected GE outbreak, or using chlorine-based products to clean up vomit. Some of the DCC characteristics that were associated previously with the presence of one of the pathogens (i.e. not stratified by sporadic and clustered cases), were not associated with clustering of those pathogens in our study, and *vice versa* (40). This indicates that they may affect the prevalence of these pathogens as a whole, but do not lead to significant clustering. Interestingly, while factors entailing transmission via staff members were previously associated with the prevalence of several enteropathogens, such as allowing staff members to work while having GE symptoms, none of them were associated with clustering of enteropathogens. We also observed that clusters of *Cryptosporidium* within DCCs were observed early in 2012, while the documented increase in *C. hominis* infections occurred in the late summer of 2012 in patients presenting to the GP, suggesting that (mainly asymptomatic) circulation of this protozoa in DCCs might have been an early indication for the increase in symptomatic cases in the community (51).

Keeping animals and having sandpits in the DCC were associated with increased clustering of *G. lamblia*. We also found that *Giardia* assemblages A and B, which are the only assemblages infecting humans, had different risk factors in DCC attendees (Chapter 7). While risk factors relating to assemblage A depicted zoonotic transmission, assemblage B was related to anthroponotic transmission. These findings add to the slowly growing body of evidence suggesting that risk factors for *Giardia* infection are indeed assemblage-specific, with assemblage A having a greater zoonotic potential than assemblage B (45, 46, 52).

Section IV: Quantifying the unexpected decrease in rotavirus laboratory detections in the Netherlands

What is already known?

Rotavirus is the leading cause of diarrheal disease in children of preschool age (53). By the age of 5 years, almost all children have acquired rotavirus infection at least once (54). Since licensing of two rotavirus vaccines (Rotarix and Rotateq) in 2006 in Europe, many countries have introduced rotavirus vaccination as part of their immunization programs, which led to substantial drops in rotavirus GE hospitalizations (55). In the Netherlands, rotavirus vaccination will become part of the immunization programme in 2019, but only for children with medical risk conditions (56). The rotavirus season in the Netherlands usually follows the one that

is typically observed in temperate climates, with annual epidemics in winter months that peak in February–March (57). Until 2014, rotavirus was responsible for 3,300 to 4,800 hospitalizations yearly in children below 5 years of age in the Netherlands (58).

In 2014, an unexpected low number of rotavirus detections was reported by laboratories registered with the Dutch Working Group for Clinical Virology (NWKV) (59). Between August 1999 and July 2013, an average annual 1,362 rotavirus detections (range 1,001–2,000) were reported (59). From August 2013 and July 2014, a significant drop by 56% was observed, with 570 rotavirus detections. In addition, the usual peak in rotavirus circulation in March had shifted to May. The weekly all-cause GE consultation rate in children below 5 years in GP surveillance had also significantly dropped, with 36% in that year, with the usual February-March peak being absent. Although lower temperatures during the 2013/2014 winter and a low proportion of susceptible children contributed to the 2014 drop, this could not fully explain it, suggesting that other unknown factors were instrumental (60). Other hypotheses included a relatively mild course of the disease due to reduced pathogenicity, or herd immunity due to rotavirus vaccination programs in neighboring countries.

What this thesis (Chapters 8 and 9) adds

In Section IV (Chapters 8 and 9), we quantified the prevalence of rotavirus in children below 5 years in the general population of the Netherlands in order to determine whether the 2014 drop was a genuine decrease in rotavirus circulation or, e.g., the result of decreased pathogenicity of the circulating strains. Moreover, using laboratory data on rotavirus detections, GE consultations from GP surveillance, and hospitalization data on GE, we described whether a hyper-endemic rotavirus year occurred following 2014 due to an accumulation of susceptible children that had not been infected in 2014, as well as a shift in the age distribution towards older children.

In Chapter 8, we found that among children in the general population that were sampled irrespectively of symptoms, the rotavirus prevalence during the usual rotavirus season (January–April) in 2014 was significantly lower (0.6%) as compared with the same period in 2010–2013 (range: 7%–11%). This showed that the 2014 drop was unlikely to be the result of a less severe course of the disease, which was supported by much lower GE consultation and hospitalization rates in children below 5 years in 2014 than in earlier years, to which rotavirus is one of the major contributors. In Chapter 9, results indicated that there was no hyper-endemic rotavirus season after 2014. In 2015, the rotavirus season was as usual, and

surprisingly the 2016 season was low once again, with significant reductions in the number of rotavirus laboratory detections and GE consultation rates compared with 2010–2013 (-59% and -35%, respectively). Similar to 2014, the 2016 rotavirus season was at its peak in May instead of March. As hypothesized, the age distribution of GE consultations in 2015 season had shifted towards older children, affecting relatively more 2- and 3-year old children. These are the first studies to describe a possible biannual rotavirus pattern in a country without rotavirus vaccination, which has only been observed before in countries with moderate to high rotavirus vaccination coverage (61-63). However, this pattern did not continue into 2018, as rotavirus laboratory detections from virological surveillance indicate that the rotavirus activity in 2017 and 2018 was similar to pre-2014 years.

Implications of this thesis for research and public health policy

Recommendations for research into preschool gastroenteritis

Community burden and costs

Because most of the GE burden occurs at the community level, data on the incidence and etiology of GE in the general population are essential to support decision-making of possible intervention measures. When estimating the incidence of GE in the general population through self-reported complaints, prospective studies produce the most accurate incidence estimates, but are much more costly and time-consuming than retrospective studies. Hence, retrospective designs dominate the literature on the community burden of GE. However, comparison of incidence rates between retrospective studies is often hampered by the use of a different recall period, which strongly influences incidence estimates. For example, the GE incidence in a study in the United Kingdom comparing a recall period of 7 days with a recall period of 28 days found the incidence to be three-fold higher in the 7 days period, with 1.5 episodes/person-year and 0.5 episodes/person-year, respectively, and similar findings were reported in the United States of America (64, 65). Therefore, future studies should carefully consider the length of the recall period to ensure comparability with previous studies, herewith being able to follow trends over time. Furthermore, our societal cost estimates for GE did not include data on premature mortality and chronic sequelae, which future cost-of-illness studies

should take into account because these generally dominate the costs associated with GE morbidity (66).

High-risk child populations

Studies identifying host factors that determine the susceptibility of pathogen-specific illness are pivotal in understanding why certain child populations are at increased risk for GE. We found that children with asthma, developmental disorders (including autism), or food allergies were at increased risk for GE. Although the literature provides several hypotheses as to why these risk groups may have an increased susceptibility to GE, e.g. changes in gut microbiome, chronic intestinal inflammation, use of certain medicines, poor hygiene behavior, etc., the reasons remain largely unknown (67-70). Comparing the intestinal microbiome of healthy individuals with these high-risk child populations may provide clues as to why they are more susceptible for GE. Moreover, it would allow us to unravel the effect of antacid use on the gut microbiome, which we identified as a risk factor for GE and has previously been found to change the gut microbiome composition in a way that would promote infection (71, 72). For this purpose, a unique dataset of the gut microbiome of 4,000 randomly selected individuals from the Netherlands' general population could be used, which were sampled as part of a recent nationwide population-based study called PIENTER-3 and will become available in 2020 (73). These data may help to increase our understanding of the role of the gut microbiome in the susceptibility to enteric infections, of which the body of knowledge has quickly expanded in recent years (72). For example, a study among Swedish travelers found that those with lower faecal microbiota diversity were more susceptible to *Campylobacter* infection (74). Understanding these determinants is essential in identifying child populations that would benefit most from therapeutic interventions or vaccination strategies.

Interpreting laboratory results

Asymptomatic carriage of one or multiple enteropathogens is common in preschool children in the Netherlands. This poses major challenges for the interpretation of laboratory results, as detection of an enteropathogen might not be clinically relevant, especially because molecular methods such as PCR are rapidly replacing traditional tests to detect enteropathogens in feces in childhood GE (75). With the introduction of PCR, positive results are much more common in asymptomatic children due to a substantial increase in analytical sensitivity. However, although

they have proven their advantages over previously used techniques such as rapid antigen detection and the ability to detect uncultivatable viruses, the interpretation of PCR results is complex and the distinction between asymptomatic carriage and the etiological cause of GE challenging (75). To aid with the interpretation of the results, establishing cut-offs based on cycle threshold values (Ct-values) of real-time PCRs (also referred to as quantitative PCRs) could potentially help (75-79). Ct-values express the amount of amplification cycles needed for a real-time PCR to consider the signal as positive, and is a surrogate for viral, bacterial, or protozoal load. In order to establish cut-offs for clinically relevant real-time PCR results, studies comparing the Ct-values of GE cases with those in a healthy control group would be needed, or cohort studies where GE cases serve as their own control by obtaining baseline sample when they are not symptomatic. For this purpose, real-time PCR results of children with and without GE from Chapter 4 (population-based) and chapter 6 (DCC-attendees) of this thesis could be used. Previously, a GP-based study in the Netherlands found significantly higher relative bacterial and protozoal loads in GE cases than controls for *Campylobacter*, *Salmonella,* ETEC, typical EPEC and *Cryptosporidium*, but not for *C. difficile*, *Shigella*/EIEC, STEC, EAEC, and *D. fragilis* (80). A large overlap in Ct-values was found for all of them except *Salmonella*, indicating that Ct-values could possibly only be useful to infer causality with GE for some pathogens. Preliminary studies on viruses mainly focused on establishing cut-off values for rotavirus and norovirus, and much less on other enteropathogens (76-79). These studies suggest a clear distinction in Ct-values between GE cases and healthy control for rotavirus, but to a lesser extent for norovirus. Importantly, cycle threshold cut-off values need to be validated for local real-time PCRs because of differences in laboratory techniques, but also because local distribution and quantification of pathogens can alter cut-off values and may be different between subpopulations. Another proposed method to simplify the interpretation of real-time PCR results is to relate Ct-values with severity of disease, often measured using the Vesikari 20-point scale and the Clark 24-point system for GE, which could also be explored (75). However, these data might not always be available to laboratories.

Rotavirus epidemiology

In order to estimate the impact of the introduction of rotavirus vaccination for children with medical risk conditions predisposing to severe or complicated rotavirus GE, it is essential to understand the underlying mechanisms of the current rotavirus seasonal pattern in order to distinguish the vaccine effect from other

potential factors. Based on previous research and on the one presented in this thesis, there is no evidence that the low endemic years in 2014 and 2016 were due to lower temperatures, decreasing birth cohorts, or a milder course of the disease (60). The hypothesis of herd immunity of rotavirus vaccination in surrounding countries of the Netherlands, however, remains unanswered. In all three neighboring countries, universal rotavirus vaccination has been implemented: Belgium in 2006, Germany in 2013, and the United Kingdom in 2013, with vaccination coverages between 48% and 93% (81-83). Currently, transmission modelling of rotavirus is ongoing to test the hypothesis of herd immunity. Moreover, this could be assessed by examining the temporal association between genotypes circulating in surrounding countries and those circulating in the Netherlands, for which genotype data from the EuroRotaNet surveillance network could be used (84). To monitor the impact of targeted rotavirus vaccination in the Netherlands, there is an urgent need for a surveillance system of rotavirus GE in hospitals focusing on children with medical risk conditions. The estimated effect on population-level is most likely too small to be detectable in the overall GE hospitalization rate (85). There are plans to set up a hospital surveillance system for rotavirus GE, but this is pending on funding from the Dutch Ministry of Health, Welfare and Sport.

Recommendations to reduce the burden of gastroenteritis in preschool children

Vaccination

One of the most effective measures to reduce the burden of childhood GE is through vaccination. The only enteropathogen for which human vaccines have been licensed in Europe is rotavirus, with vaccine effectiveness ranging from 78% to 93% (55, 86). In countries that introduced universal rotavirus vaccination, major reductions in rotavirus hospitalizations have been observed (55). In the Netherlands, rotavirus is scheduled to be introduced in the immunization programme in 2019, but only for children with medical risk conditions such as prematurity, low birth weight, and severe congenital pathology (85). It is estimated to avert nearly all rotavirus-related mortality, but its impact on the burden of GE in the general pediatric population is expected to be limited, with 3% reduction in rotavirus GE episodes and 15% reduction in rotavirus hospitalizations (85). The first dose should be orally administered after 6 weeks of age and the last dose no later than 24 (Rotarix) or 32 weeks (Rotateq), with minimal intervals of 4 weeks between doses (two doses for Rotarix and three for Rotateq) (86). The tight age restrictions of rotavirus vaccination

and commonly interfering medical issues of the target population make delivery of the vaccine challenging. Preliminary results of a pilot study in thirteen hospitals in the Netherlands indicate suboptimal vaccination coverage of 60% in the target groups, ranging from 39% to 81% between hospitals (87). Vaccination coverage was lowest in academic hospitals due to transferal of the child to a hospital not participating in the study. Universal rotavirus vaccination should be considered when the coverage among the target group remains low, and has the potential of reducing the rotavirus burden in the child population by more than 50%, and would avert 75% of rotavirus hospitalizations (85). However, to become cost-saving, it would require the current price of the rotavirus vaccine to be halved. Moreover, the increasing vaccine-hesitancy in parents should be carefully considered before adding a new universal vaccine to the Dutch national immunization programme.

For some other enteropathogens, such as norovirus, significant progresses have been made towards the development of a human vaccine and are currently undergoing human clinical trials, but none of them is licensed for use (88-90). Together, these could potentially reduce the morbidity and mortality due to GE substantially, for which available pathogen-specific burden estimates could be used to identify target populations that would have the greatest potential economic and health benefits to determine vaccination strategies (91).

Household transmission

Our results indicate that transmission of enteropathogens occurs extensively within households, especially in families with children below 5 years of age, causing high healthcare-seeking behaviors and productivity losses. We could not differentiate between household transmission and parallel infection from a common (third) source due to our cross-sectional design. However, a recent prospective cohort study of GE in households with children attending DCCs in Germany found that transmission from preschool child to their parents occurs in 2 out of 10 episodes and from parents to their children in 1 out of 10 episodes (13). Although some level of household transmission is inevitable, some subgroups of households are at particularly high risk of GE and might benefit from education on how to prevent transmission. These include families with children younger than 5 years that have the following characteristics: young parents, multiple children below 5 years of age, parents with a low educational level (primary, lower vocational or lower secondary education), or children with developmental disabilities. Education on basic hygiene could be done during family visits at the children's health clinic (in

Dutch: consultatiebureau), which monitors the health and development of children younger than 5 years of age in the Netherlands.

Food safety

Ensuring microbiological food safety is essential to minimize foodborne infections, which are responsible for a substantial part of the GE burden (91). We found that GE was more common in households with imperfect food hygiene practices, such as delays in food refrigeration, buying meat directly from farmers, and infrequent fridge cleaning, warranting that increased awareness of the importance of food hygiene practices is needed. Avoidance of high-risk foods that are more often or more likely to be microbiologically contaminated is particularly important in young children, those that are immunocompromised due to disease or medication, or have chronic enteropathies such as Crohn's disease (92). Examples are raw or undercooked meat, poultry, seafood, milk , eggs or sprouts (92).

The most important foodborne enteropathogens causing GE in the Netherlands include *Campylobacter*, norovirus and *Salmonella* (91). While food contamination with *Campylobacter* and *Salmonella* is mostly from animal origin, that of norovirus is often due to an infected individual. The downward trend in the number of human salmonellosis cases in Europe since 2008 has come to a halt in recent years, and is mirrored by a similar trend in laying hens. This warrants further investigations of determinants of the reversal of the decreasing trend, such as improvements of surveillance and reporting of human salmonellosis, relaxation of *Salmonella* control measures at primary production, or deficiencies in enforcing legislations by competent authorities. Recently, this topic has made its way into the strategic research agenda of the One Health European Joint Programma (EJP) (93). These data will hopefully provide and explanation for the reversal trend and provide targets for intervention measures to further reduce the *Salmonella* burden in humans.

Proton-pump inhibitors

The common use of gastric antacids, particularly PPIs, is a reason for concern. They decrease the gastric acidity that plays an important role in the defense against ingested microorganisms, hereby increasing susceptibility to bacterial pathogens (94, 95). Gastric antacids are prescribed yearly to almost 2 million patients in the Netherlands, mainly for treatment of acid-related upper gastrointestinal disorders and peptic ulcer disease, and is the most commonly prescribed drug by Dutch GPs (16% of patients) (96, 97). Because it is also available as over-the-counter medicine

without prescription, the true numbers are likely higher. The use of gastric antacids has been associated with increased infection risk of several bacteria, such as *C. difficile*, *Salmonella, Campylobacter*, and invasive strains of *E. coli* (94, 98). Moreover, it has been associated with profound changes in the gut microbiome, likely because more bacteria survive the reduced acidity of the stomach, which could be linked to increased risk for enteric infections (71, 99). Although the impact of gastric antacids on the risk of GE might be modest, its widespread use makes its effect at population level prominent (71). Therefore, although for many patients gastric antacids are an invaluable and effective treatment for several gastric acid-related disorders, the risks and benefits should be considered before initiating gastric antacids therapy, especially for those at high risk for enteric infections. These include young children, persons with comorbidities, hospitalized patients using antibiotics and those travelling to high-risk countries for GE (98). The Dutch College of General Practitioners (NHG) advices to regularly evaluate whether gastric antacids use is still needed to avoid possible side-effects (95).

Concluding remarks

Major improvements in sanitation and hygiene led to a huge reduction in diarrheal illnesses in high-income countries. Although the motto "no guts, no glory", which referred to mass causalities among war troops because of diarrheal illnesses in the 18th century, might no longer be applicable to the Netherlands, the battle against enteropathogens is far from being won. To date, the societal burden of GE is still substantial, especially in families with young children, where it would not be inappropriate to say that you need "guts" to stay healthy. Molecular studies are unravelling the ability of microbes to evolve, adapt and develop drug resistance. Moreover, they find new niches and vehicles for infection, showing important changes in their epidemiology over the past decades. Cutting-edge scientific advances continue to elucidate the complex mechanisms by which enteropathogens can cause diarrhea, and have identified new targets for research. For instance, there is increasing appreciation that most microbes in our environment are not pathogenic per se, and that the gut microbiome plays an important role in health and disease.

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APPENDIX

Summary

Gastroenteritis (GE) is an inflammation of the gastrointestinal tract that is often caused by infectious agents. GE is characterized by diarrhea and/or vomiting, and is a leading cause of morbidity and mortality worldwide. In industrialized countries like the Netherlands, GE is rarely life threatening, but it causes a significant burden in terms of societal costs due to its high frequency of occurrence, particularly in children below five years of age. This thesis aims at improving our understanding of the aetiology, epidemiology and burden of GE in preschool children and their parents/caretakers in the Netherlands.

In **Chapter 2**, we estimated the incidence of GE in the Netherlands' general population to be 0.8 GE episodes/person-year during 2014–2016 based on a population-based repeated cross-sectional study. The incidence of GE was particularly high in children younger than 5 years of age and in adults living in households with these children. The annual societal costs due to GE were estimated at €945 million, mainly due to loss of productivity (i.e. paid worktime missed) and hospitalization. In **Chapter 3,** we used epidemiological data from child-parent pairs that were sampled at random from the Dutch general population to characterize GE risk in households with preschool children. In children, risk factors for GE were having chronic enteropathies, chronic respiratory diseases, or developmental disabilities, but also gastric antacid use, non-breastfeeding, toddling age, parental occupation in healthcare, having multiple siblings, and living in single-parent families. Day-care centre (DCC) attendance was a risk factor for GE, but only up to 12 months of attendance, possibly indicating that they may acquire some early immunity to GE afterwards. Risk factors for GE in parents were female gender, younger age, having multiple or developmentally disabled DCC-attending children, antimicrobial use, gastric antacid use, and a number of poor food handling practices.

In **Chapter 4,** we determined the prevalence and clinical relevance of a range of potentially GE-causing viruses, bacteria, and protozoa, and identified risk factors for infection with these enteropathogens in preschool children and their parents based on stool sample testing from the same child-parents pairs of Chapter 3. A substantial overlap of enteropathogens between children and parents suggested significant household transmission, which was particularly evident for viruses. Viruses predominated in winter, bacteria in summer, while protozoa had a less pronounced seasonal pattern. Most infectious agents were detected just as much in children and parents with GE as in those without GE, indicating that not all enteropathogen

infections may be clinically relevant. In preschool children, norovirus genogroup II (GII), astrovirus, and adenovirus type 41 were significantly associated with GE, and in parents, these were norovirus GII, sapovirus and *Cryptosporidium*. In **Chapter 5**, we showed that preschool children with co-infections of viruses, bacteria, and/or protozoa do not have an increased risk for GE as compared with children with single infections.

In **Chapter 6,** we presented risk factors for temporal clustering of the main enteropathogens contributing to GE morbidity in DCCs in the Netherlands, which almost half of Dutch preschool children attend. These were rotavirus, norovirus, astrovirus, *Giardia*, and *Cryptosporidium*. DCCs with playground sandpits more often had clusters of these enteropathogens, possibly due to contamination of sandpits with these pathogens. Hygiene-related factors were associated with decreased clustering of some of the pathogens, such as extra cleaning of toys during a suspected GE outbreak, or using chlorine-based products to clean up vomit. Interestingly, while factors entailing transmission via staff members were previously associated with the prevalence of several enteropathogens (i.e. not stratifying by sporadic and clustered cases), such as allowing staff members to work while having GE symptoms, none of them were associated with clustering of enteropathogens. This indicates that they may affect the prevalence of these pathogens as a whole, but do not lead to significant clustering. Additionally, in **Chapter 7,** we showed that risk factors for infection with *Giardia* assemblage A in DCC attendees were related to zoonotic transmission, while those for assemblage B infection were related to anthroponotic transmission, a finding that add to the growing body of evidence suggesting that assemblage A has a greater zoonotic potential than assemblage B.

In **Chapters 8** and **9**, we reported a significant decrease in the rotavirus prevalence among preschool children (sampled irrespectively of symptoms) in the Netherlands in 2014, indicating a genuine decrease in rotavirus circulation in absence of rotavirus vaccination. Although a hyper-endemic year was expected in the following year, the number of rotavirus laboratory detections in 2015 was as usual and, surprisingly, once again low in 2016. However, the age distribution of GE consultations in 2015 season had shifted towards older children, affecting relatively more 2- and 3-year old children. Although the emergence of a possible biannual rotavirus pattern was hypothesized, the number of rotavirus laboratory detection in 2017 and 2018 were similar to pre-2014 years. To date, the underlying mechanisms of the rotavirus pattern remain largely unknown.

In **Chapter 10,** we discuss what the research presented in this thesis adds to the current body of knowledge. We also discuss the public health implications of our findings and give recommendations for future research and recommendations to reduce the burden of GE in preschool children in the Netherlands. These were as follows:

Recommendations for research into GE during preschool years

- Retrospective studies estimating the GE incidence should carefully consider the recall period that they use, as this strongly influences incidence estimates, enabling comparison with incidence estimates from previous retrospective studies.
- Societal cost estimations for GE should also include data on premature mortality and chronic sequelae because they are major drivers of costs associated with GE morbidity.
- Comparing the intestinal microbiome of healthy individuals with high-risk child populations for GE (e.g. children with asthma, developmental disorders, and food allergies) may provide clues as to why they are more susceptible for GE.
- Studies should further explore whether it is possible to make a distinction between asymptomatic carriage of infectious agents and the etiological cause of GE based on cycle threshold values of the real-time polymerase chain reaction (PCR). This would aid in the interpretation of PCR results, which is currently complex due to its high sensitivity.
- A remaining hypothesis that could explain the rotavirus pattern that emerged in 2014 in the Netherlands is potential herd immunity due to rotavirus vaccination in surrounding countries. For this purpose, mathematical transmission models could possibly provide answers, or studies assessing the temporal association between rotavirus genotypes in the Netherlands and surrounding countries. For the latter, data from the EuroRotaNet surveillance network could be used.
- A surveillance system should be set up of rotavirus GE in hospitalized children with medical risk conditions, including prematurity, low birth weight, and severe congenital pathology, in order to monitor the impact of targeted rotavirus vaccination in the Netherlands, which will be implemented in 2019.

Recommendations to reduce the burden of GE during preschool years

- Universal rotavirus vaccination in children below 5 years of age could be considered when the vaccination coverage among the target group using targeted rotavirus vaccination remains suboptimal.
- Households that would benefit the most from education on how to prevent household transmission are families with children younger than 5 years with the following characteristics: young parents, multiple children below 5 years, parents with a low education level, or children with developmental disabilities. This could be done during family visits at the consultation bureaus.
- Increased awareness of food hygiene practices within families is needed to minimize foodborne infections, as well as avoidance of high-risk foods that are more likely to be microbiologically contaminated, especially for young children, persons who are immunocompromised, and those with chronic enteropathies.
- Clinicians should consider the risks and benefits before initiating gastric antacid therapy, especially for those at high risk of enteric infections, including young children, persons with comorbidities, hospitalized patients using antibiotics, and those travelling to high-risk countries for GE.

Samenvatting

Gastro-enteritis (GE) is een ontsteking van het maagdarmkanaal die meestal wordt veroorzaakt door infectieuze agentia. Het wordt gekenmerkt door diarree en/of braken en is wereldwijd één van de voornaamste oorzaken van ziekte en sterfte. In geïndustrialiseerde landen zoals Nederland is GE zelden levensbedreigend, maar het zorgt voor aanzienlijke maatschappelijke kosten vanwege de hoge frequentie van voorkomen, met name bij kinderen jonger dan vijf jaar. Dit proefschrift heeft tot doel om meer inzicht te verschaffen in de etiologie, epidemiologie en ziektelast van GE bij kinderen jonger dan vijf jaar en hun ouders/verzorgers in Nederland.

In **Hoofdstuk 2** werd de incidentie van GE in de Nederlandse bevolking geschat op 0,8 GE-episoden/persoonsjaar in de jaren 2014–2016. Dit werd gedaan op basis van data uit een herhaaldelijk uitgevoerde cross-sectionele studie naar GE in de algemene bevolking. We vonden de hoogste incidentie bij kinderen jonger dan 5 jaar en volwassenen met jonge kinderen in het huishouden. De jaarlijkse maatschappelijke kosten als gevolg van GE werden geraamd op € 945 miljoen, wat voornamelijk toe te schrijven was aan productiviteitsverlies (het missen van betaalde werktijd) en ziekenhuisopname. In **Hoofdstuk 3** werden epidemiologische gegevens gebruikt van willekeurig geselecteerde kind-ouder koppels uit de Nederlandse bevolking om het risico op GE in huishoudens met jonge kinderen in kaart te brengen. Risicofactoren voor GE bij kinderen waren chronische darmziekten, chronische luchtwegaandoeningen, ontwikkelingsstoornissen, het gebruik van maagzuurremmers, het niet krijgen van borstvoeding, een leeftijd van 13–36 maanden, ouders die werkzaam zijn in de gezondheidszorg, het hebben van meerdere broers en zussen en wonen in een éénoudergezin. Ook naar het kinderdagverblijf (KDV) gaan was een risicofactor voor GE, maar niet voor kinderen die langer dan 12 maanden naar het KDV gingen. Dit komt mogelijk door het ontwikkelen van immuniteit voor GE. Risicofactoren voor GE bij ouders waren vrouwelijk geslacht, jongere leeftijd, het hebben van meerdere KDV-gaande kinderen of kinderen met ontwikkelingsstoornissen, antibioticagebruik, het gebruik van maagzuurremmers en een aantal factoren gerelateerd aan voedselbereiding en veiligheid.

In **Hoofdstuk 4** beschreven we de prevalentie en klinische relevantie van meerdere potentieel GE-veroorzakende virussen, bacteriën en protozoa, evenals risicofactoren voor infectie bij kinderen jonger dan vijf jaar en hun ouders. Dit werd gedaan door middel van fecesonderzoek bij dezelfde kind-ouder koppels als hoofdstuk 3. We vonden een aanzienlijke overlap van enteropathogenen bij kinderen

en hun ouders, hetgeen suggestief is voor significante overdracht van pathogenen binnen het huishouden, voornamelijk van virussen. Virussen overheersten in de winter, bacteriën in de zomer, terwijl protozoa een minder uitgesproken seizoenspatroon hadden. De meeste pathogenen werden net zo vaak gevonden bij kinderen en ouders met GE als kinderen en ouders zonder GE. Dit geeft aan dat niet alle infecties met enteropathogenen klinisch relevant zijn. Bij kinderen onder de vijf jaar waren norovirus genogroup II (GII), astrovirus en adenovirus type 41 significant geassocieerd met GE. Bij ouders waren dit norovirus GII, sapovirus en *Cryptosporidium*. In **Hoofdstuk 5** vonden we dat kinderen jonger dan vijf jaar met co-infecties van virussen, bacteriën en/of protozoa geen verhoogd risico hadden voor GE in vergelijking met kinderen met infecties van één pathogeen.

In **Hoofdstuk 6** beschreven we risicofactoren voor clustering (het vaker voorkomen in een bepaalde tijdsperiode dan verwacht) van enteropathogenen in KDV's in Nederland. Bijna de helft van de Nederlandse kinderen jonger van vijf jaar gaan naar het KDV. We beschreven de pathogenen die de grootste bijdrage leveren aan de GE-ziektelast in KDV's, namelijk rotavirus, norovirus, astrovirus, *Giardia* en *Cryptosporidium*. KDV's met zandbakken hadden vaker clusters van deze enteropathogenen, mogelijk als gevolg van verontreiniging van zandbakken met deze pathogenen. Een aantal hygiëne-gerelateerde factoren waren geassocieerd met verminderde clustering van sommige pathogenen, zoals extra reiniging van speelgoed tijdens een vermoedelijke GE-uitbraak of het gebruik van chloorproducten om braaksel op te ruimen. In een eerdere studie was de prevalentie van enkele enteropathogenen geassocieerd met factoren die transmissie via personeelsleden suggereerden. Echter, geen van deze factoren was geassocieerd met clustering van deze enteropathogen in onze studie. Dit geeft aan dat ze mogelijk wel invloed hebben op het vóórkomen van deze pathogenen, maar niet leiden tot significante clustering. In **Hoofdstuk 7** vonden we dat risicofactoren voor infectie met *Giardia*-assemblage A bij kinderen op het KDV gerelateerd waren aan zoönotische transmissie, terwijl die voor assemblage B-infectie gerelateerd waren aan mens-op-mens transmissie. Deze bevinding draagt bij aan het toenemende bewijs dat assemblage A een groter zoönotisch potentieel heeft dan assemblage B.

In **Hoofdstuk 8 en 9** beschreven we een significante afname van de prevalentie van rotavirus bij kinderen jonger dan vijf jaar (geselecteerd onafhankelijk van symptomen) in 2014 in Nederland. Dit was opvallende omdat er in Nederland niet voor rotavirus wordt gevaccineerd. Hoewel in het opvolgende jaar een hyper-endemisch jaar werd verwacht was het aantal positieve laboratoriumtesten

voor rotavirus in 2015 zoals gebruikelijk en, verrassend, opnieuw laag in 2016. De leeftijdsverdeling van huisartsconsulten voor GE was echter wel verschoven naar oudere kinderen in 2015, met relatief meer consulten onder 2- en 3-jarige kinderen. Hoewel de opkomst van een mogelijke tweejaarlijks rotaviruspatroon werd verondersteld, was het aantal positieve laboratoriumtesten voor rotavirus in 2017 en 2018 vergelijkbaar met het aantal vóór 2014. Tot op heden blijven de onderliggende mechanismen van de huidige rotavirus epidemiologie grotendeels onbekend.

In **Hoofdstuk 10** bespraken we wat het onderzoek in dit proefschrift toevoegt aan de huidige kennis en wat de gevolgen zijn voor de volksgezondheid. Daarnaast gaven we aanbevelingen voor toekomstig onderzoek en aanbevelingen om de ziektelast van GE in kinderen jonger dan vijf jaar in Nederland te verminderen. Deze waren als volgt:

Aanbevelingen voor onderzoek naar GE bij kinderen jonger dan vijf jaar

- Retrospectieve studies die de GE-incidentie schatten moeten zorgvuldig overwegen welke recall-periode gebruikt wordt, aangezien dit de incidentieschattingen sterk beïnvloedt. Hiermee wordt het vergelijken van incidentieschattingen met eerdere retrospectieve onderzoeken mogelijk.
- Schattingen van maatschappelijke kosten voor GE zouden ook gegevens over vroegtijdige sterfte en chronische gevolgen mee moeten nemen, omdat ze verantwoordelijk zijn voor een groot deel van de kosten.
- Het vergelijken van het darmmicrobioom van kinderen met een verhoogd risico voor GE, zoals kinderen met astma, ontwikkelingsstoornissen en voedselallergieën, met het darmmicrobioom van gezonde kinderen kan inzicht geven in waarom zij vatbaarder zijn voor GE.
- Er is behoefte aan onderzoek naar het maken van onderscheid tussen asymptomatisch dragerschap van infectieuze agentia en de etiologische oorzaak van GE op basis van de drempelwaarde (Ct waarde) van de real-time polymerase chain reaction (PCR), wat zou helpen bij de interpretatie van PCR-resultaten.
- Een hypothese die de huidige epidemiologie van rotavirus in Nederland zou kunnen verklaren is potentiële kudde-immuniteit als gevolg van rotavirusvaccinatie in omringende landen. Mogelijk kunnen wiskundige transmissiemodellen hierop antwoord geven, of studies die de temporele associatie tussen rotavirusgenotypen in Nederland en omringende landen bestuderen. Voor laatstgenoemde kunnen gegevens van het surveillancenetwerk EuroRotaNet worden gebruikt.

- Met de geplande invoering van rotavirusvaccinatie bij prematuren, kinderen met een laag geboortegewicht en kinderen met een aangeboren afwijking, is behoefte aan een surveillance systeem voor GE veroorzaakt door rotavirus bij deze doelgroep om de impact van rotavirusvaccinatie te kunnen monitoren.

Aanbevelingen om de GE ziektelast te verminderen bij kinderen jonger dan vijf jaar

- Universele rotavirusvaccinatie bij kinderen jonger dan 5 jaar kan worden overwogen wanneer de vaccinatiegraad onder de huidige doelgroep voor rotavirusvaccinatie (prematuren, laag geboortegewicht, aangeboren afwijking) suboptimaal blijft.
- Huishoudens die de meeste baat hebben bij voorlichting over het voorkómen van pathogeenoverdracht binnen het huishouden zijn gezinnen met kinderen jonger dan 5 jaar met de volgende kenmerken: jonge ouders, meerdere kinderen jonger dan 5 jaar, ouders met een laag opleidingsniveau of kinderen met ontwikkelingsstoornissen. Dit kan gedaan worden tijdens gezinsbezoeken aan het consultatiebureau.
- Er is meer aandacht nodig voor voedselhygiëne in gezinnen om het aantal voedselinfecties te verminderen. Ook is het vermijden van voedingsmiddelen die een verhoogd risico hebben op microbiologisch besmetting van belang, voornamelijk bij jonge kinderen en personen met een verminderde immuniteit of chronische darmziekten.
- Clinici zouden de voor- en nadelen van maagzuurremmers af moeten wegen voordat gestart wordt met behandeling. Dit is vooral van belang bij personen met een verhoogd risico op darminfecties zoals jonge kinderen, personen met comorbiditeiten, ziekenhuispatiënten die antibiotica gebruiken en personen die een hoog-risico land voor GE bezoeken.

Dankwoord

Zo, daar zijn we dan. Het dankwoord. Het laatste wat ik schrijf, waarschijnlijk het eerste wat u leest. Vijf jaar geleden, in 2014, begon ik als naïeve junior epidemioloog bij het RIVM. Drie jaar later, in 2017, mocht ik als buitenpromovendus "beginnen" aan de Universiteit Utrecht. In de afgelopen jaren ben ik altijd met plezier naar werk gegaan, waarmee ik mij gelukkig prijs. Het was een tijd waarin mijn passie voor het vak is gegroeid en ik veel heb geleerd. Uiteraard heb ik dit traject niet in mijn eentje afgelegd. Ik ben geholpen en geïnspireerd door velen om mij heen, die ik graag wil bedanken.

Allereerst mijn copromotoren. Wilfrid, ik heb geluk gehad met jou als leidinggevende. Je passie voor het vak was aanstekelijk. Het eerste jaar dacht ik wel eens, "Maar is het nou nog niet goed?", als jij mij wederom vroeg een analyse op een andere manier te proberen. Nu snap ik dat die wetenschappelijke nieuwsgierigheid, het 'struinen door de data', nieuwe inzichten geeft en waardevol is. Ik struin onderhand heel wat af in data. Heerlijk. Daarnaast leek je oplossend vermogen en creativiteit oneindig. Bedankt voor de vrijheid die je mij gaf en dat ik deel mocht uitmaken van de gastro-groep. Lapo, een groot deel van wat ik nu weet heb ik geleerd van jou. Je deur stond altijd open, waar ik veelvuldig gebruik van heb gemaakt. Je bent de man van de track-changes, niet gesloten do-files en creatieve titels. Mede door jou weet ik nu dat de wereld groter is dan Nederland (en helemaal niet zo eng…). Niet alleen als collega was je waardevol, ik beschouw je als een goede vriend. De vele fietstochten hebben hieraan bijgedragen, zelfs al moest ik af en toe (lees: altijd) tot na de afgesproken tijd wachten. Gaaf dat ik dit dankwoord nu in het Nederlands kan schrijven!

Ook wil ik mijn promotoren bedanken. Roel, twee jaar geleden liep ik bij je binnen met een stapeltje papieren. Of je mijn promotor wilde zijn. Bedankt dat je mij deze mogelijkheid hebt gegeven. Je scherpzinnigheid, tijdige commentaar en pragmatisme heb ik erg gewaardeerd. Jaap, ik was blij dat ook jij mijn promotor wilde zijn. Bedankt voor de overleggen en feedback op mijn artikelen en inleiding en discussie van dit proefschrift. Uiteraard ook voor de gezelligheid. Je oprechte interesse en belangstelling stelde ik op prijs.

Graag bedank ik de leden van de commissie voor hun bereidheid om mijn proefschrift te beoordelen. Ook bedank ik alle coauteurs voor het meedenken en meeschrijven aan de artikelen. In het speciaal Daan, Harry en Titia; jullie input op de discussie van dit proefschrift werd gewaardeerd.

Natuurlijk mijn collega's van de GEZ groep. Jullie deur stond altijd open. Bedankt voor al jullie antwoorden op mijn vragen en de gezelligheid, zeker bij de barbecues. Ingrid, jou wil ik speciaal bedanken. Zeker in mijn begintijd bij het RIVM heb je mij enorm geholpen met veel van de werkzaamheden die voor mij nieuw waren. Ook Barbara, jij hebt mij in het eerste jaar op weg geholpen. Eelco, bedankt voor de vrijheid die ik kreeg/krijg in mijn werkzaamheden en de flexibiliteit om mijn proefschrift af te ronden.

Kamergenoten Mariëtte en Renske. Mariëtte, mijn vaste kamergenootrots in de branding. Hoewel je maar één dag per week op het RIVM was, vond ik het leuk dat we samen een passie deelde voor 'pielen met data'. Bedankt ook voor het in leven houden van de plant. Zelfs met jouw reminders vergeet ik dat ding. Renske, bedankt voor de erg gezellige tijd en goede gesprekken (nog steeds!). Door jou ben ik bekend geraakt met de gouden standaard van kwalitatief onderzoek, de KAP studie. Je zou het eens moeten proberen.

Lieve vrienden en familie, bedankt voor jullie interesse, gezellige afleiding en stabiele basis. Lieve broer en zus, fijn dat jullie mijn paranimfen, of paranymfo, of hoe het ook heet, willen zijn.

Manon, wat ben jij een lieverd. Fijn om met jou alle grote stappen te maken. Huisje (Herenweg), boompje ("tak" in de tuin), beestje (fluffy Pip) én recent een klein lief manneke. Heerlijk om na een lange werkdag thuis te komen in een warm huis. Dat geldt figuurlijk, maar ook letterlijk. Geen zorgen, ik heb je defecte interne thermostaat allang geaccepteerd. Bedankt dat je mij altijd steunt. Op naar meer leuke avonturen!

Curriculum Vitae

Roan Pijnacker was born on March 13th, 1989 in Woerden, the Netherlands. After graduating from secondary school he studied Medical Radiation Technology at the InHolland University of Applied Sciences in Haarlem, where he obtained his Bachelor's degree in 2011. While working part-time as radiation therapist in the Amsterdam University Medical Center (formerly Amsterdam Medical Center), he pursued a master in Health Sciences with a specialization in Infectious Diseases and Public Health at the VU University in Amsterdam. Upon completion, he started working as an epidemiologist at the department for Epidemiology and Surveillance at the National Institute for Public Health and the Environment (RIVM) in 2014. Whilst working here, he completed a two-year fellowship with the European Programme for Intervention Epidemiology Training (EPIET). He was employed twice as short term field epidemiologist with Doctors Without Borders in South Sudan to plan and conduct a mortality and nutrition survey and investigate a rabies outbreak. In 2017, he started his PhD at Utrecht University on the basis of work he had already undertaken and published. He continues his current position as epidemiologist at the RIVM.

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