On norovirus outbreaks and transmission in hospitals

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2019

Document Version:
Publisher's PDF, also known as Version of record

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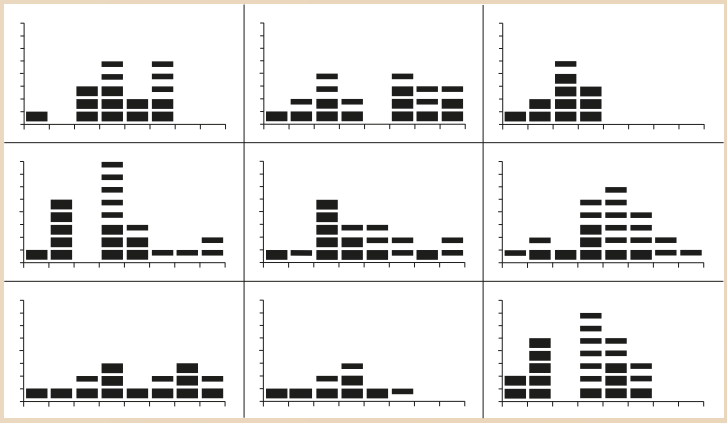
FACULTY OF MEDICINE | LUND UNIVERSITY

Printed by Media-Tryck, Lund 2019 NORDIC SWAN ECOLABEL 3041 0903



Epidemic curves over the first eight days from examples of norovirus outbreaks at hospital wards in Skåne. Black cubicles are new patient cases, black/white are healthcare worker cases.

Noroviruses are the leading cause for gastroenteritis worldwide. The high infectivity of noroviruses results in frequent and disruptive outbreaks in healthcare facilities. In this thesis some aspects of norovirus outbreaks and transmission in hospitals are investigated.

LUND UNIVERSITY

FACULTY OF MEDICINE

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2019:98
ISBN 978-91-7619-827-8
ISSN 1652-8220

ON

NOROVIRUS OUTBREAKS

AND

TRANSMISSION

IN HOSPITALS

Carl-Johan Frænkel

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Belfragesalen, Biomedical Center, Lund, at 13:00 on the 25th of October 2019

Faculty opponent
Professor Johan Westin

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Organization LUND UNIVERSITY	Document name **DOCTORAL DISSERTATION**
	Date of issue 25th of october 2019
Author: Carl-Johan Frænkel	Sponsoring organization

Title and subtitle: On norovirus outbreaks and transmission in hospitals

Abstract

Noroviruses are now the leading cause of gastroenteritis worldwide. Noroviruses are effectively transmitted due to a low infectious dose, viral shedding in high concentrations, environmental stability, and they induce only a limited immunity after infection. Especially, the norovirus variants of genotype II.4 (GII.4) have seemed greatly adapted for pandemic spread. The high infectivity of noroviruses result in frequent outbreaks, particularly at healthcare facilities. Hospital norovirus outbreaks cause excess morbidity among vulnerable inpatients, ill staff, shortage of beds and economic loss.

In the first study, using two outbreak surveillance methods, based on either clinical reporting or clustering of positive laboratory results, incidence of norovirus outbreaks at all hospital wards in Skåne was estimated to 0.5 to 0.2 per ward and winter season, 2010-2012. In total, 135 outbreaks were detected, 74 with both methods, 18 only by clinical reporting and 43 only with laboratory surveillance. Laboratory surveillance performed better at identifying outbreaks and seem a stable option for continuous surveillance, but the combination of both surveillance methods would be preferred.

In the second study, the first patient in each outbreak (n 65), was compared with patients not involved in any outbreak (n 186), to analyse factors associated with outbreak development. Sharing room with other patients, old age, comorbidities, onset of disease at the ward and vomiting were independently associated with an outbreak outcome. Infection with norovirus GII.4 was associated with outbreak development and vomiting, suggesting a possible explanation for the efficient transmission of GII.4 in hospitals.

In a third study, aspects of possible airborne transmission was investigated, by analysis of air samples collected in the proximity of 26 norovirus patients. Norovirus genome in the air was a common finding during outbreaks, supporting the hypothesis that airborne transmission might be of importance. The size of the norovirus carrying particles and the concentration of norovirus genomes in air allow for the possibility of airborne transmission. A short time period since the last vomiting event in the room was associated with norovirus positive air samples, indicating vomiting as the major source of airborne norovirus.

In the final study, aspects of environmental transmission was explored. Different exposures related to room admission and association to norovirus acquisition at the ward were analysed. A room stay at a room with a prior occupant with norovirus infection was a small but independent risk factor for acquisition of norovirus infection. Norovirus acquisition was not observed in association to sharing room with patients with recently resolved symptoms of norovirus infection.

In conclusion, prevention of hospital outbreaks may be improved by better recognition of factor associated with outbreak development and improved surveillance. Airborne transmission and dissemination of norovirus, originating from vomiting events, may be an important mode of transmission, but further studies are needed. Environmental transmission from prior room occupants stress cleaning and disinfection as important preventive measures.

Key words: Norovirus, outbreak, transmission, prevention, surveillance, epidemiology

Classification system and/or index terms (if any)

Supplementary bibliographical information Faculty of Medicine Doctoral Dissertation Series 2019:98		**Language: English**
ISSN and key title 1652-8220		**ISBN 978-91-7619-827-8**
Recipient's notes	**Number of pages**	Price
	Security classification	

Signature  Date 2019-09-19

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Cover by Clara Fraenkel

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ISBN 978-91-7619-827-8
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2019

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MADE IN SWEDEN

We have no right to limit the way in which a disease may be propagated, for the communicable diseases of which we have a correct knowledge spread in very different manners

John Snow (1813-1858)

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Infection Control and Hospital Epidemiology. 2017 Jan;38(1):96-102.

II. **Risk factors for hospital norovirus outbreaks: impact of vomiting, genotype, and multi-occupancy rooms.**

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III. **Sources of Airborne Norovirus in Hospital Outbreaks.**

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Clinical Infectious Diseases. 2019 Jun 29. (Epub ahead of print)

*Carl-Johan Fraenkel and Malin Alsved contributed equally to this manuscript.

IV. **Risk of acquiring norovirus infection from prior room occupants and roommates.**

Fraenkel Carl-Johan, Böttiger Blenda, Malin Inghammar.

Manuscript in preparation.

Abbreviations

CI 95%	Confidence interval of 95 %
Ct	Cycle threshold
CVID	Combined variable immunodeficiency
FCV	Feline calicivirus
HCW	Healthcare worker
HSCT	Hematopoietic stem cell transplantation
HBGA	Histo-blood group antigen
ICT	Infection control team
ID50%	Infectious dose for 50 % of exposed
IQR	Inter quartile range
LTCF	Long term care facility
NoV	Norovirus
OR	Odds ratio
ORF	Open reading frame
P	Probability value
PCR	Polymerases chain reaction
R_{eff}	Effective reproduction number
RT-PCR	Reverse transcriptase PCR
RR	Rate ratio
SOT	Solid organ transplant
VP1	Viral protein 1, major capsid protein
VP2	Viral protein 2, minor capsid protein

Thesis at a glance

Paper	Objective	Method	Result
Paper I	To determine how common norovirus outbreaks are at Swedish hospitals by using an comparing two survaillance systems	All outbreak reports included in the clinical surveillance of hospitals in Skåne were compared to a clusterdefinition based on laboratory surveillance of norovirus test results and admission date 2010-2012.	135 outbreaks at the 194 wards were identified during the two winter seasons. Incidence varied between 0.5-0.2 outbreaks/ward and season. Laboratory surveillance outperformed clinical surveillance, but a combination is the best.
Paper II	To explore factors that trigger outbreak development in association to individual norovirus patients	A nested case-control study comparing 65 outbreak index cases with 186 sporadic cases	Sharing room, vomiting and high age and co-morbidity of the NoV patient were all associated with outbreak development. Infection with norovirus GII.4 was also associated with outbreak and with vomiting.
Paper III	To explore the possibility of airborne transmission of norovirus	Collection of air in the proximity of norovirus patients, in relation to symtoms and outbreaks. The size of norovirus-carrying particles was aslo investigated	24 % of all airsamples were positive for norovirus. All positive airsamples were obtained from patients in ongoing or coming outbreaks, implying a possible connection. The particles were small enough to travel with air currents and in concentration able to cause disease.
Paper IV	To investigate the risk of acquiring norovirus from a prior room occupant or roommates with resolved norovirus infection	Cohort study of all patients admitted to 5 Infectious Disease wards 2013-2018, comparing those exposed to a potentially norovirus contaminated room with non-exposed.	There is a small risk of acquiring norovirus if assigned to a room with a prior norovirus infected occupant, inferring that cleaning is important. Sharing a room with a patient with resolved symptom (since > 48 hours) seems safe.

Sammanfattning på svenska

Vinterkräksjuka, orsakad av norovirus, är nu den enskilt vanligaste orsaken till infektiösa diarrésjukdomar i hela världen. Man beräknar att norovirus orsakar ca 700 miljoner sjukdomsfall, 220 000 dödsfall och kostar det globala samhället cirka 600 miljarder SEK årligen.

Norovirus är väldigt smittsamt, endast några få virus krävs för smitta samtidigt som det i ett gram avföring kan finnas 10 miljarder viruspartiklar. Dessutom är viruset stabilt i miljön och är svår att avdöda med desinfektionsmedel. Det verkar inte heller som att immunförsvaret alltid kan skapa något långvarigt skydd mot nya norovirus infektioner. Att vi sett en sådan ökning av norovirusinfektionerna sedan mitten av 1990-talet beror på att noroviruset sedan dess lyckats skapa nya virusvarianter med jämna mellanrum. Dock verkar virusutvecklingen avstannat de sista åren, men det är oklart om det bara är tillfälligt.

På sjukhus orsakar norovirus stora problem genom sin smittsamhet. Utbrotten drabbar främst de redan sköra patienterna, som kan tvingas skjuta upp viktiga operationer eller cancerbehandlingar. Norovirusinfektionen leder ofta till förlängd sjukhusvård och ibland till allvarliga komplikationer. Dessutom drabbas ofta många i personalen och de redan få sjukhusplatserna blir ännu färre. Utbrotten är dessutom ofta svåra att stoppa effektivt. Det vore därför önskvärt att förhindra att utbrotten startar överhuvudtaget.

I den här avhandlingen har olika aspekter på sjukhusutbrott studerats och olika smittvägar undersökts, i förhoppningen att ny kunskap ska kunna leda till ett mer effektivt förebyggande arbete.

I det första delarbetet undersöktes två metoder för att identifiera sjukhusutbrott. Dels klinisk rapportering till den vårdhygieniska enheten, dels övervakning av positive laboratoriesvar från patienter med norovirusinfektion. Totalt hittades 135 utbrott på Skånes 194 avdelningar under vintersäsongerna 2010-2011 och 2011-2012. Av alla avdelningar drabbades 30 % av minst ett utbrott under 2010-2011 och räknar man bara medicinavdelningarna, drabbades 43 %. Antalet drabbade avdelningar var lägre under vintern 2011-2012 som var ett, ur ett norovirus perspektiv, lugnare år. Övervakningen av laboratoriesvar visade sig vara bättre än klinisk övervakning.

I delarbete II undersöktes vilka faktorer som är avgörande för att ett utbrott ska starta. Patienter som hade gett upphov till ett utbrott, eller i alla fall var det första

fallet i utbrottsundersökningen, jämfördes med patienter med norovirusinfektion där det inte blev något utbrott efteråt. Det visade sig att om patienten delar rum med andra patienter, är äldre (över 80 år), har annan sjuklighet, insjuknar på avdelningen eller kräks, så ökar risken för att det ska bli ett utbrott. Att kräkning visade sig vara en riskfaktor är extra intressant eftersom detta dessutom var vanligare vid infektion med de virustyper som tidigare visat sig kunna sprida smitta extra effektivt. Kanske är det kräkningen som är nyckeln till att norovirus kan sprida sig så framgångsrikt i sjukhusmiljö?

I delarbete III undersöktes aspekter på eventuell luftsmitta av norovirus, bland annat i relation till kräkning. Luftprover samlades in i närheten av patienter med norovirusinfektion och proverna analyserades för att kunna upptäcka spår av norovirus i luften. Norovirus kunde påvisas i totalt 21 av 86 luftprover (24 %). Om patienten hade kräks inne på rummet var sannolikheten större att luftprovet innehöll norovirus, 64 % av alla luftprover som samlades in inom tre timmar från en kräkning var positiva. Koncentrationen var också i de flesta fall tillräcklig för att möjliggöra smitta genom att andas in luften under, som minst, 10 minuter upp till ett par timmar. Smitta förutsätter dock att virus i luften motsvarar aktiva virus partiklar. När storleken på virusbärande partiklar i luften mättes kunde det konstateras att partiklarna är så små att de kan hålla sig kvar i luften länge och föras vidare med luftströmmar. Eftersom norovirus även kunde hittas i avdelningskorridorerna visar det också att smittan kan spridas utan för patientrummen. Bara luftprover insamlade under utbrott var positiva, med ett enda undantag. I detta fall blev det faktiskt ett utbrott några dagar senare. Denna observation skulle kunna tolkas som att norovirus i luft har betydelse för smitta och utbrottsutveckling.

I delarbete IV undersöktes aspekter på smitta från miljön genom att studera om det innebär en risk att som patient bli placerad i ett rum där det nyligen legat en patient med norovirusinfektion, trots att rummet städats. Det visades sig faktiskt vara en liten extra risk att bli placerad på ett sådant rum. I de statistiska beräkningarna uppskattades det att av 187 patienter som placeras i ett rum där det inom den senaste veckan legat en patient med norovirus infektion, insjuknar en (1) på grund av kvarvarande smitta i miljön. Däremot insjuknade ingen av de 54 patienter som delade rum med någon som nyligen varit sjuk i vinterkräksjuka.

Avslutningsvis verkar det som att man med relativt enkla metoder, på ett bra sätt kan övervaka sjukhusutbrott av norovirus. Bättre kunskap om de faktorer som styr utbrottsutvecklingen kan hjälpa det vidare förebyggande arbetet. Luftsmitta av norovirus behöver undersökas närmare, men att viruset iallafall verkar finnas i luften och att luftsmitta är möjligt gör att vi kan behöva fokusera mer på sjukhusventilationen. Städningen behöver dessutom bli bättre för att ytterligare förebygga smitta från miljön.

Preface

I encountered my first norovirus outbreak in Växjö 1998. I was forced into one last night shift when nearly all my colleagues at the Department of Infectious Diseases fell ill during one evening after eating the cut salad at the hospital staff restaurant. Later, working with infection control in Blekinge, these outbreaks became more prevalent and suddenly I and the Infection Control team were supposed to help curb the elusive chains of norovirus transmission. When I came to Lund 2010, everybody just wanted to discuss one single infections control question: How can we stop the norovirus outbreaks? Because they were everywhere. Most of the previous scientific reports were based on single outbreak observations – but in Skåne there was an opportunity to collect massive data, and I began collecting with the help of the Infection Control team in Skåne. Transmission has since, thankfully, calmed down, but the future is, as always, uncertain.

During the latter part of writing this thesis I have become inspired by the pioneering work of Dr John Snow. In the mid-19th century, he traced the source of the London cholera outbreaks, before the discovery of the cholera bacteria. By use of modern epidemiologic methods (and some anecdotes), he concluded that cholera is mainly transmitted by contaminated water and not, as was the prevailing hypothesis at the time, by foul air (effluvia or miasma). I have included some quotes from his book "On the mode of communication of cholera" to highlight some similarities and dissimilarities between the understanding of the cholera epidemic in London 1855 and norovirus today.

Introduction

Outline of the history of norovirus

In 1929 dr Zahorsky described a disease characterized by vomiting and diarrhoea, primarily observed during the winter months which he called “winter vomiting disease”, or “hyperemesis hiemis” [1]. No causative organism was detected, but it might have been norovirus. In 1935 a large epidemic of nausea, vomiting and diarrhoea occurred in Roskilde, described by the Danish physician dr Henningsen [2]. This outbreak was probably caused by norovirus and winter vomiting disease is still called “Roskildesyge” in Denmark.

The discovery of the virus was made following an investigation of an outbreak of gastroenteritis in an elementary school in Norwalk, Ohio, USA, in 1968 [3]. In just two days, 50 % (116/232) of the students and teachers at the school developed nausea, vomiting and abdominal cramps, persisting for 12-48 hours, symptoms resembling the illness earlier described by dr Zahorsky. The secondary attack rate of family members was 32 %. Volunteers challenged by ingestion of filtrated bacteria-free stool samples originating from the Norwalk outbreak, developed typical gastroenteritis symptoms [4]. Kapikian *et al.* were able to visualise virus particles using electron microscopy in the stool of the ill volunteers [5]. The virus was called Norwalk virus. Similar viruses were subsequently called Norwalk-like viruses or simply “small round structured virus”.

With a method to diagnose the disease, it now became apparent that Norwalk-like viruses accounted for a large proportion of gastroenteritis outbreaks [6]. Since the electron microscopy detection method was expensive and time consuming, a set of clinical criteria for establishing outbreak causation by Norwalk-like viruses was introduced, the Kaplan criteria: 1) negative stool culture, 2) ≥ 50% of cases with vomiting, 3) mean duration of illness 12-60 hours and 4) (if available) incubation period of 24-48 hours [7].

The genome of the Norwalk-like viruses was characterised 1990 [8]. The genetic information made the introduction of PCR-based diagnostic methods possible [9]. With improved diagnostics Norwalk-like viruses were now recognised as the leading cause of epidemic gastroenteritis. [10, 11].

Multiple attempts have been made to cultivate noroviruses since the Norwalk outbreak without success, a struggle constituting a major obstacle to achieve better control strategies. The breakthrough came in 2016, when Ettayabi *et al.* were able to cultivate noroviruses for the first time [12]. The cultivating system is complicated but the method is spreading [13].

The Norwalk-like viruses were renamed *Norovirus* 2002. The same year, concurrent with the introduction and pandemic spread of a new norovirus variant, a sharp increase of norovirus gastroenteritis was observed, with large and frequent outbreaks [14]. In Europe the outbreaks primarily target semi-enclosed facilities, such as hospitals, nursing homes, schools and day-care centres [14].

Noroviruses are now the leading cause for sporadic and outbreak gastroenteritis worldwide across all age-groups [15]. Winter vomiting disease is today globally responsible for an estimated 700 million gastroenteritis episodes, 220,000 deaths and costing the society 60 billion USD in health system costs and productivity loss [16]. Winter vomiting disease has become a well-known and, due to its infectivity, sometimes feared illness, occasionally referred to as a modern plague. Or cholera.

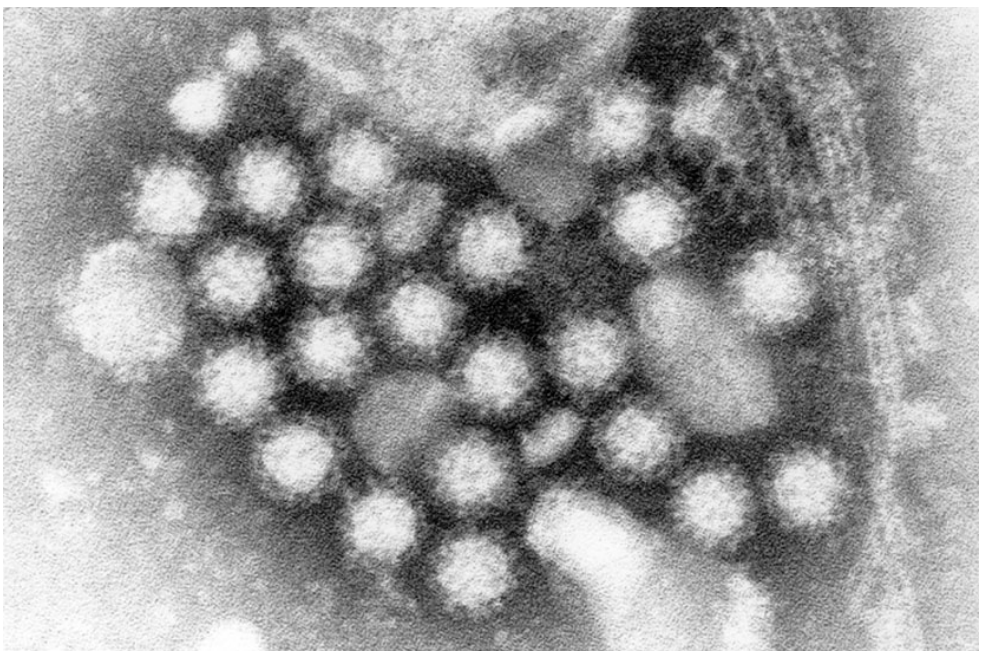

Figure 1.Electromicroscopy of noroviruses.Photo Kjell-Olof Hedlund. By permission of Folkhälsomyndigheten.

Norovirus

Structure

Norovirus particles are non-enveloped and have a diameter of about 27-40 nm (Figure 1). The genome is composed of a linear single-stranded, positive-sense RNA of about 7.600 bases in length. In the human noroviruses there are three open reading frames (ORFs) encoding eight viral proteins [17]. ORF-1 encodes the non-structural proteins, including an RNA-dependent RNA polymerase. ORF-2 and ORF-3 encode the major capsid protein VP1 and the minor capsid protein VP2, respectively (Figure 2). The VP1 proteins are arranged together to form the icosahedral symmetry of the virion and form what look likes goblet-shaped depression (calyces) on the surface, and hence the name of the virus family. The VP1 protein can be divided into the shell (S) and the protruding C-terminal (P). The S domain is highly conserved and forms an inner shell around the viral RNA genome. The P domains, divided into the P1 and P2 subdomains, forms protrusions on the capsid surface [18]. The P2-domain, which is protruding furthest from the surface, is variable and also flexible and are thought to interact with attachment receptors, contains neutralizing antibody epitopes and evolves under immune selection pressure [18, 19]. The VP2 proteins are thought to arrange themselves to a portal through which the viral genome can be delivered into a new host cell and thereby initiate infection [20].

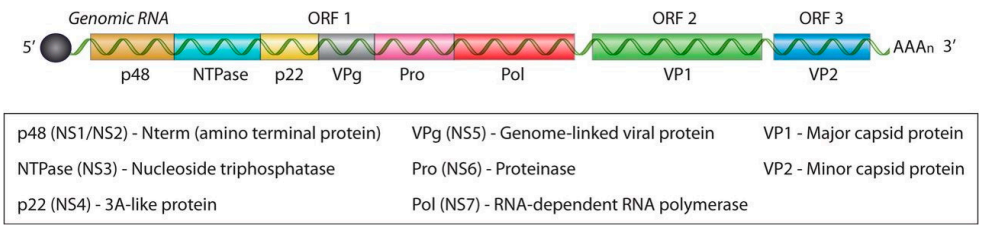

Figure 2. The human norovirus genome. The genome is comprised of a linear, positive-sense RNA, ~7.6 kb in length, covalently linked to the viral protein genome (VPg) (solid black circle) at the 5′ end and polyadenylated at the 3′ end. There are three open reading frames (ORFs), designated ORF-1, ORF-2, and ORF-3, encoding 8 viral proteins. ORF-1 encodes the 6 nonstructural (NS) proteins that are proteolytically processed by the virally encoded cysteine proteinase (Pro). ORF-2 and ORF-3 encode the structural components of the virion, viral protein 1 (VP1) and VP2, respectively. Reprinted with permission from Robilotti *et al,* Clin. Microbiol. Rev. 2015. Copyright © 2015, American Society for Microbiology.

Phylogeny

Caliciviridae family

The *Norovirus* genus belong to the *Caliciviridae* family. Of the now five genera included in the *Caliciviridae* family only the *Sapovirus and Norovirus* genera contains virus strains that can cause human disease. Sapovirus, previously named Sapporo-virus, cause similar symptoms as noroviruses but is less prevalent [21]. Sometimes sapoviruses and noroviruses are collectively referred to as "calicivirus". In scientific literature, calicivirus often refers to feline (cat) calicivirus (FCV), another member of the *Caliciviridae* family [22].

Genogroups

The *Norovirus* genus can be divided and classified into at least 10 genogroups based on phylogenetic analysis of the virus genome [23, 24]. Genogroup I, II, IV and VIII and IX (recently reclassified from GII.15) contain strains that can infect humans (Figure 3). The absolute majority of cases derive from GII and a smaller part from GI while infections with other genogroups are rare. Genogroup III, V, VI, VII, X infect various animal species. The GV genogroup, rodent (murine) norovirus, is together with FCV the most common surrogates for human norovirus in experimental research as they can be readily cultivated in laboratories. [25].

Genotypes

Genogroups are further divided into genotypes, primarily based on the sequence of the VP1-gene. There are currently 30 genotypes that are known to infect humans, 9 GI genotypes (GI.1-9), 18 GII genotypes (GII.1-10, GII.12-14, GII.16-17, GII.20-22) and one genotype of each GIV, GVIII and GIX genogroup [23, 24]. Genotype GI.1 refers to the virus of the original Norwalk outbreak. The most successful genotype, the GII.4 genotype, has dominated human norovirus disease in the last 20 years, and is the cause for the modern norovirus epidemic spread [11].

GII.4 variants

Genotypes can be even further subdivided into variants. Variants are best recognized for GII.4. The variants are recognized by genetic drift resulting in amino-acid differences primarily at the P2-domain of the VP1 protein, and thereby often change viral receptor affinities and the immune response. All RNA viruses have a high mutation rate, but not all mutations are fit for effective transmission and replication [26]. The GII.4 genotype seems to be more prone to recombination and mutation of the variable protruding P2-domain than other noroviruses and thereby being better able to respond and adapt to herd immunity of the population [19, 27, 28]. New GII.4 variants have emerged and spread in pandemic epidemics every last 2-3 years, causing increased outbreak activity in hospitals and other semi-enclosed settings, as

well as in the community (Figure 3) [19, 29, 30]. But, no entirely new pandemic GII.4 strain has emerged since 2012. The recent emergence of GII.17 genotype variants are also spreading, mostly in Asia, and future will tell if this genotype will be able to evolve and adapt in a way similar to the GII.4 genotype [31].

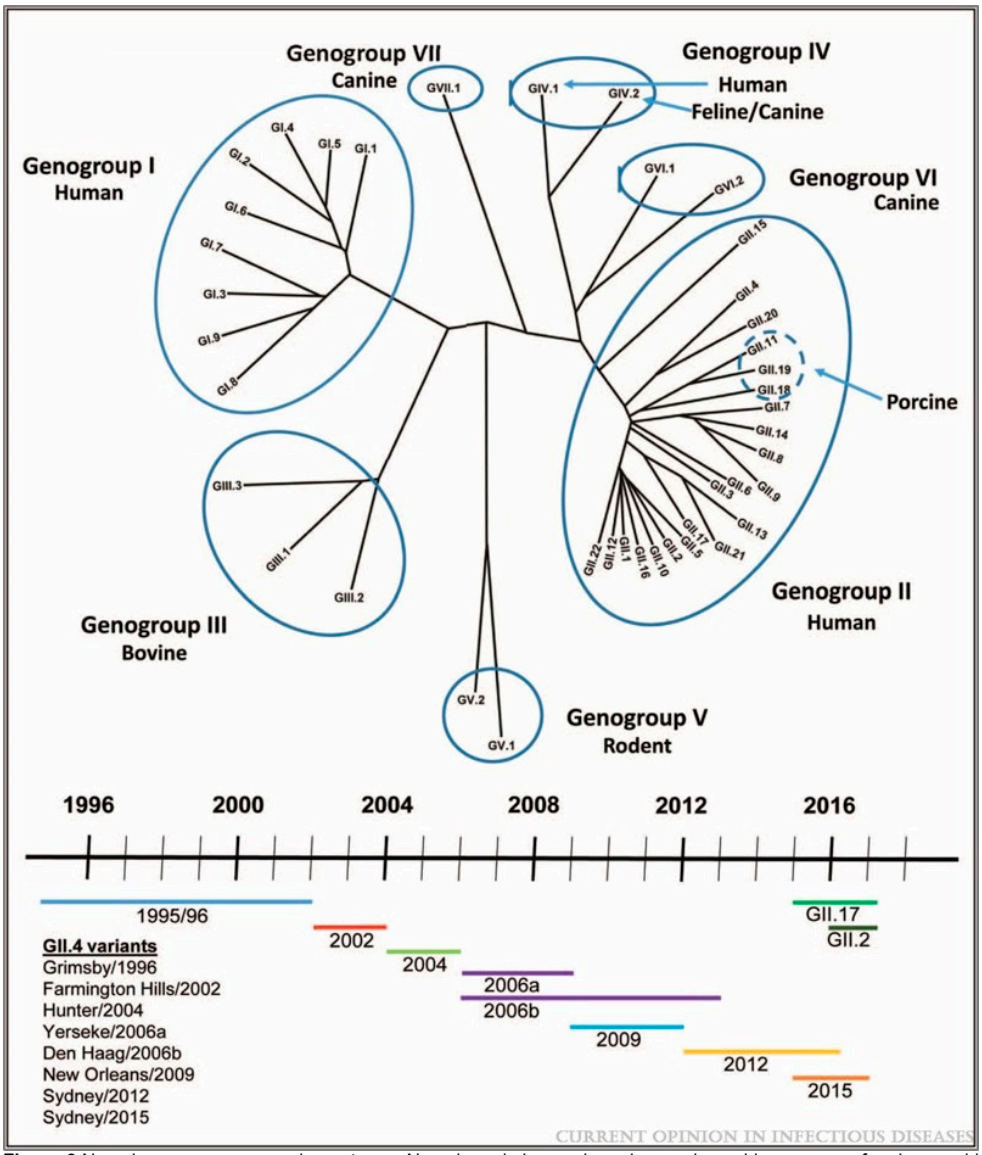

Figure 3. Norovirus genogroups and genotypes. Norovirus phylogeny based on amino acid sequence of major capsid protein (VP1) (a) and timing of emergence of GII.4 variants and other recent genotypes (b). Part (a) reprinted with permission of Cortes-Penfield et al, Clin Ther. 2017; © 2017Elsevier HSJournals and part (a-b) of Atmar *et al,* Curr Opin Infec Dis 2018; © 2018 Wolter Kluwer Health.

Note: The phylogenetic classification was recently updated. For the newest classification, see text or ref [24].

Norovirus infection

Pathogenesis

The pathogenesis of human noroviruses involves a variety of interactions between different human host cells and bacteria in the gut microbiome. Despite recent major advances, many key aspects of virulence, symptom induction and host susceptibility remain unknown.

The intestinal epithelial cells, enterocytes, of the small intestine (duodenum, jejunum and ileum) seem to be the primary site of infection [30]. One key to successfully develop the in-vitro culture system was virus replicating in enterocytes [12, 32]. Biopsies from immunocompromised patients with chronic infection found histopathological evidence of infection in the small intestine, with villus flattening, oedema and inflammatory infiltration of the lamina propria and intercellular presence of the virus in enterocytes of small intestine [33]. Diarrhoea is mainly driven by leak flux as a result of barrier dysfunction and a secretory component [34]. The significance of the involvement of inflammatory cells and gut microbiome in pathogenesis is still uncertain [35, 36]. The enteric nervous system is hypothesised to play a role in vomit induction and diarrhoea since serotonin is released, at least in rotavirus gastroenteritis, and pharmacological inhibition seems to reduce symptoms [37, 38].

Immunity

Genetic susceptibility

Histo-blood group antigens HBGAs are complex carbohydrates, glycans, expressed on the outer surface of specific cells, including enterocytes and red blood cells, and are present in different secretions, including saliva. The synthesis of the HBGAs is mediated by enzymes controlled by the FUT2 (secretor), FUT3 (Lewis) and ABO(H) genes [39]. Noroviruses can bind to different HBGAs and the attachment is important for infection progression. Different norovirus genotypes have different affinity to different HBGAs [19]. In a society, subpopulations with a specific set of HGBAs thus have reduced susceptibility to some norovirus genotypes. Individuals with an inactivated FUT2 enzyme, non-secretors, lack HBGAs in their intestinal mucosa and secretions and rendering resistance to several (but not all) norovirus genotypes, including the predominant GII.4 [40, 41]. About 15-20 % of the populations in Europe are non-secretors [42]. Non-secretors are not just protected from norovirus gastroenteritis, but also from asymptomatic viral shedding. Also the ABO blood group HBGAs, independent of FUT2, may affect the risk of contracting norovirus gastroenteritis, as individuals with blood type B have a partial protection

against certain norovirus strains [43]. The importance of Lewis antigen is less certain [39].

Immune response

After the Norwalk outbreak challenge studies were performed where volunteers were repeatedly exposed to the virus. Exposure after just 4-8 weeks after the first infection did not result in a new symptoms, but repeated exposure 2-4 years later, resulted in new symptomatic infections [44]. Another challenge study concluded that the immunity lasted about 6 months [45]. In these studies the infecting dose was very high and the relevance to natural infection has been questioned. In a more recent modelling study the duration immunity was estimated to be 4-8 years [46]. The immunity is mainly conferred by IgG and/or IgA antibodies that can block the HBGA binding site [47-49]. The antibody immune response is also important for infection resolution and viral clearing. Immune deficiencies affecting the antibody producing B-cells often result in chronic infection [50, 51]. The immunity is generally genotype or variant specific, but it seems that after a number of different norovirus infections during childhood, cross-reactive antibodies that protect also against closely related genotypes are produced [52, 53]. These findings imply that after some initial norovirus infection a substantial part of the adult population is immune to several of the circling genotypes [28]. This herd immunity increases the evolutionary pressure on the norovirus strains, and if a strain is able to mutate or recombine to a new fit variant that can evade the immune response, a novel pandemic strain is born [54]. The GII.4 genotype has achieved this at least six times in the past 20 years, but not since 2012. Future will tell if the so far successful genotype will accomplish this again.

Vaccine development

In spite of the many remaining questions regarding duration, cross-reactivity and serologic marker of protective immune response, vaccine development is moving forward. At least five vaccine candidates are currently being evaluated [23]. Two vaccines have progressed to clinical studies, both have been shown to be safe and immunogenic in Phase 1 studies [55]. One of them, an intramuscularly administered bivalent vaccine (GI.1 and GII.4), has completed an initial Phase II study with some promising results, at least in reducing illness severity [56-58].

Clinical manifestations

Incubation period

Kaplan *et al.* pooled data from 38 outbreaks and found the incubation period to be 24-48 hours [6]. A more recent analysis of pooled data of outbreak reports and challenge studies estimated the median incubation period to be 1.2 days (~ 29

hours), with an interquartile range (IQR) of 0.9-1.7. In five percent of the individual observations the incubation period was less than 0.5 days and more than 2.6 days, respectively [59]. The incubation period seems inversely related to the inoculum dose, with an incubation period in the longer range for low-dose exposure [60].

> "The period which intervenes between the time when morbid poison enters the system, and the commencement of the illness which follows, is called the period of incubation. It is, in reality, a period of reproduction, as regards the morbid mater; and the disease is due to the crop or progeny resulting from the small quantity of poison first introduced. In cholera, this period is much shorter than in most other epidemic or communicable diseases...., it is shown to be in general only from twenty-four to forty-eight hours."
>
> John Snow, "On the mode of communication of cholera", 1855.

Infectious dose

The infectious dose is low, but how low has been of some controversy. Noroviruses have a tendency to group themselves and depending on how to account for virus aggregation the result is different. The conclusion after two challenge studies is that the human infectious dose infection 50 % of those exposed (ID50%) is 18 virus particles, or genomic equivalents, (CI 95% 1-4350) for disaggregated viruses [61, 62]. For aggregated viruses the ID50% is 1300-2800 genomic equivalents, depending on host susceptibility [60]. Recently, it was discovered that noroviruses are shed as viral clusters, aggregated inside a vesicular envelope. The formation may stay intact during faecal-oral transmission, which might result in enhanced infectivity and virulence and might affect the *in vivo* infectious dose [63].

Symptoms of norovirus infection

Winter vomiting disease caused by human noroviruses is characterized by sudden onset of nausea, vomiting, followed by abdominal cramps and watery diarrhoea [64]. Low grade fever is seen in about a third of the cases [3, 65, 66]. Malaise, myalgia, chills and headache may be associated symptoms. Duration of illness is normally short in immunocompetent hosts, lasting one to three days (mean two days) [66, 67]. Usually vomiting, fever and other constitutional symptoms are seen just the first day [65]. About 15-30 % of the cases experience only vomiting and no diarrhoea and 20-35 % only diarrhoea and no vomiting [66, 68, 69]. The panorama of norovirus infection span from asymptomatic excretion of virus to (rarely) life threatening disease.

Symptoms and age

Data suggests differences in clinical manifestations between different age-groups. In children vomiting tend to be of more frequent symptom, reported in about 80 % of the cases versus diarrhoea in 50-70%, as are constitutional symptoms [68, 69]. In adults, diarrhoea is more pronounced, reported in about 70-85 % versus vomiting in 60-70% [66, 67]. In the elderly above 80 years of age, there are even less

constitutional symptom and less frequent vomiting and more often diarrhoea is the only symptom [67]. High age correlates to longer duration of symptom, as the mean duration of symptoms for patients aged >80 years was 5.7 days compared with 3.7 days for those aged <80 years in a hospital outbreak reported by Partridge *et al* [70]. Patients involved in hospital outbreaks have a significantly longer duration of illness, median 3-4 days [67, 70].

Symptoms and genotype

Some data also indicate an association between norovirus genotype and symptoms. Several studies has suggested that GII.4 variants cause a more severe disease than other genotypes, and thus would be more virulent. In a nursing home study in the Netherlands 2005-2007, outbreaks with GII.4 was associated with higher incidence of vomiting among the residents, but not among the staff (Table 1) [71]. Duration of illness is also reported to be longer in GII.4 illness in residents of long-term care facilities [72].

Table 1. Symptoms reported during norovrius outbreaks in nursing homes by genotype, separate for residents (n 637) and staff (n 390).

		GII.4 (%)	Other (%)	OR[a]
Residents				
	Diarrhea	82.8	85.5	0.8 (0.5-1.4)
	Vomiting	**57.8**	**41.6**	**1.9 (1.3-2.9)**
	Diarrhea and vomiting	**43.3**	**29.2**	**1.9 (1.2-2.9)**
	Nausea	**50.4**	**35.3**	**1.9 (1.1-3.1)**
	Stomach ache	29.9	22.4	1.5 (0.8-2.7)
	Abdominal cramps	**23.5**	**9.4**	**3.0 (1.3-6.5)**
	Fever	**19.2**	**7.1**	**3.1 (1.3-7.6)**
	Mucus in stool	7.3	0	p= 0.009
	Numer of symtoms: median	2.0	1.0	p< 0.001
Staff				
	Diarrhea	85.7	78.4	1.7 (0.7-3.7)
	Vomiting	78.7	80.0	0.9 (0.4-2.0)
	Diarrhea and vomiting	71.3	65.3	1.3 (0.7-2.5)
	Nausea	81.8	81.5	1.0 (0.1-2.4)
	Stomach ache	41.6	49.2	0.7 (0.4-1.4)
	Abdominal cramps	61.0	66.2	0.8 (0.4-1.6)
	Fever	18.2	30.8	0.5 (0.2-1.1)
	Mucus in stool	3.4	1.5	2.6 (0.3-25.6)
	Numer of symtoms: median	3.0	3.0	p=0.21

Reproduction (truncated) of table from Friesema *et al, J Clin Virol 2009 [71].*

In a systematic review GII.4 strains were associated with a 9-fold increased risk of hospitalisation and a 3-fold increase risk of death compared to other genotypes [73]. In a recent study reporting combined data from 3747 US norovirus outbreaks 2009-2016, GII.4 outbreaks was associated with a higher hospitalisation ratio (RR 1.54;

95% Cl, 1.23-1.96) and a higher mortality (RR 2.77; 95% Cl 1.04-5.78) versus non-GII.4 outbreaks. It was also noted that the last two GII.4 variants, New Orleans (2009) and Sydney (2012) seemed to be associated with fewer hospitalisations and lower mortality as compared to the preceding Den Haag genotype (2006).

Infections in immunocompromised patients

Patients with severe immunosuppression, including patients with solid organ transplant (SOT), hematopoietic stem cell transplant (HSCT) and common variable immunodeficiency (CVID) have a higher risk for contracting severe norovirus infection with prolonged or chronic diarrhoea. This may result in malnutrition, graft dysfunction and an increased risk of death [51]. Sixteen percent of HSCT patients developed severe and prolonged infection in a Swedish case series [74]. In a cohort of 65 renal transplant patients with norovirus infection, 40 % presented with acute transplant failure [75]. Patients with CVID have a high risk of developing chronic diarrhoea and shedding of norovirus for many years [50, 76].

Complication

Complications associated with norovirus infections are generally infrequent. Dehydration, electrolyte abnormalities and renal insufficiency are the most common complications seen. Some individuals have an increased risk of complicated course of illness. Apart from immunocompromised patients also infants, elderly, and patients with co-morbidities have a higher risk of a complications and a higher mortality. Neonates risk developing, the still rare, norovirus-associated necrotizing enterocolitis, which is accompanied with a high mortality [77, 78]. Seizures, both febrile and afebrile are a known complication to norovirus infection in children less than five years of age [79, 80]. Patients with cardiovascular disease are at increased risk of electrolyte disturbances which might lead to arrhythmias [81]. Rare complications such as oesophagus rupture and bowel perforation are also reported [82-84]. In reports stating the cause of death associated with norovirus infection aspiration pneumonia, gastroenteritis or sepsis are the most common [85].

Mortality

Globally the 70,000-220,000 estimated total annual number of deaths associated with norovirus infection are mostly attributed to infants in low-income countries and elderly [15, 16, 86]. The global mortality associated with norovirus infection is estimated to 3/100,000 infections [87]. In United States noroviruses cause an average of 570-800 deaths and in England and Wales about 80 deaths per year [88, 89]. The risk of death, not surprisingly, increases with old age. Hall *et al* reported mortality rates of 0.02/100,000 for the age group 5-64 years to 2/100,000 person-years above 65 years [90]. In a study of mortality associated with norovirus outbreaks in American nursing homes a significant 11 % increase in all-cause

mortality was observed during outbreaks compared to non-outbreak periods [91]. A Swedish study also found increased mortality in elderly patients hospitalised for community-onset norovirus gastroenteritis compared to similar patients already hospitalised [92].

Another aspect of norovirus associated mortality is the risk of misinterpretation of vomiting, of other causes, as probable norovirus infection. Vomiting is a non-specific symptom, often seen in diseases with a much higher mortality, for example sepsis. Fear of transmission might result in less medical attention.

Asymptomatic infection

In outbreak investigations and norovirus challenge studies some exposed individuals do not develop symptoms of norovirus gastroenteritis, but still excrete noroviruses in their stool. This fraction just developing an asymptomatic infection is often up to a third of the exposed [93, 94]. Non-secretors on the other hand are usually negative for norovirus in faecal samples, even after virus ingestion [39, 95]. The combined effect of non-secretor status and acquired immunity in many situations may add up to almost half of an exposed adult population being protected and consequently you rarely see outbreak reports with an attack rate above 50-60 % [96]. Exactly why some develop symptoms of infection and others do not is incompletely understood. Some individuals exposed in challenge studies develop asymptomatic infection without having any measurable protective antibodies or known genetic resistance [97]. These findings highlight the knowledge gaps concerning protective serologic correlates.

Viral shedding

Noroviruses are shed in high concentration during and after the symptomatic period. The concentration in diarrhoeal samples are approximately $10x\ 10^{10}$ virus copies per gram faeces and $10x10^5$ virus copies per millilitre emesis [98-101]. After a norovirus infection virus is excreted in stool for some time after the symptoms have resolved. In challenge studies the duration of shedding has been between 1-3 weeks. The viral load gradually decreased after a peak about four days after inoculation, usually after the symptoms have resolved. [98, 102]. In hospital outbreaks some patients, especially elderly, immunocompromised, and infants may shed virus for longer periods [99, 103-105]. Individuals with asymptomatic infection shed virus in about the same concentration and duration as symptomatic patients [99]. Pre-symptomatic shedding a few hours before symptom onset has also been observed [98].

Shedding of norovirus in asymptomatic individuals is common, especially in children less than five years of age. A seasonal variation is also seen [106]. The prevalence of a positive norovirus faecal test in asymptomatic individuals range from 0-36% in the reported studies [107]. A high prevalence is more commonly

observed in low-income countries, but an English study reported an age-adjusted prevalence of 12 % [106]. The only Swedish study, performed at children's day care centres, reported a prevalence of just 0.7 % (3/438) [108]. Asymptomatic shedding may be of importance to hospital transmission, and we will return to this topic further on in this thesis.

Diagnosis

After the discovery of the Norwalk-virus, visualisation of virus particles by immune electron microscopy was used for diagnosis. The method required expensive equipment, skilled microscopists and often took several days to perform, hence establishing a confirmed diagnosis was mostly confined to outbreak investigations.

Detection

Now diagnosis rely on reverse transcriptase polymerase-chain reaction (RT-PCR) were the RNA genome of the virus first is transformed to cDNA and then detected with repeated replications using primers. With real-time RT-PCR (qPCR) the process can be further automated and the result enables an estimation of the virus concentration in the sample. The higher the number of replication cycles needed to detect the virus at the cycle threshold (Ct), the lower is the concentration. The sensitivity and specificity of the RT-PCR assays used are very high [109].

Still the primers have to correspond the virus genes. The diversity and evolution of noroviruses can make this a challenge and periodic updating of the assays is recommended [110]. Most assays use the conserved region at the ORF1-ORF2 polymerases-capsid junction for primer target and separate primers for detection of GI and GII genogroups, respectively [111].

The preferred sample for norovirus detection is stool but vomit samples can also be analysed. Rectal swabs may be used instead of stool sample [112]. Norovirus can also be found in mouthwash after vomiting [113].

Sequencing

If information about genotype or variant is required sequencing of the genome must be performed. A variable part of the genome gives the most information and hence the ORF-2 with the variable P-domain is often the best choice. With the information of the exact RNA sequence, a phylogenetic tree can be constructed by genetic comparison of previous strains and there by retrieve the genotype and variant of the analysed strains [114]. Occasionally, sequencing of the polymerases gene is performed, in that case the genotype is depicted with a P.

Treatment

Treatment of norovirus gastroenteritis is merely based on rehydration with fluids and electrolytes. Oral rehydration is preferred but intravenous fluids are sometimes necessary if vomiting is intense or dehydration is severe [64]. Antimotility agents can be used to decrease diarrhoea temporarily. Antiemetic drugs, specifically 5-HT antagonists (ondansetron) have been shown to shorten and reduce symptoms of viral gastroenteritis in children [38]. Nitazoxanide inhibits norovirus and have been tried as treatment from immunocompromised patients with chronic infection and in a small randomized trial with promising results, but also failures are reported [115-117]. Ribavirin and oral or intravenous immunoglobulin have also been tried with some success[118].

Norovirus epidemiology

Norovirus is now the leading gastroenteritis pathogen and account for almost a fifth of all cases of gastroenteritis worldwide [15]. Norovirus incidence varies in different populations, age groups, by year and by season. Norovirus infections occur both as sporadic cases and in outbreak events.

Incidence

It is difficult to establish a correct incidence since norovirus infection is usually a self-limiting disease and only a fraction of the ill seek medical attention, and even fewer are sampled. Some relevant incidence studies are presented in Table 2. The incidence in Sweden is not known but if British and Dutch reports are translated the result would be around 400.000 ill cases and 1400 hospitalised patients per year.

Table 2. Estimations of the incidence of norovirus illnesses, outpatients visists, hospitalisations and deaths per 10.000 inhabitants.

	Illnesses /10,000	Outpatient visits /10,000	Hospitalisations /10,000	Deaths /10,000	Year	ref
USA	650	64	2.4	0.03	1996-2007	[88, 119]
England	450 470	54 21 49	 7[1]		1993-1996 2008-2009 2007-2013	[120] [121] [122]
The Netherlands	380	92	1.2	0.04	2009	[123]
Europe	641			0.02		[87]
Global	995			0.3		[87]

[1] Including admission and discharge diagnosis.

The incidence is highest among children younger than 5 years. In a recent British cohort study during 2008 and 2009 the overall incidence was 47 cases per 1000 person-years, but 143 cases per 1000 person years for the age group younger than five years (Table 3) [124].

Table 3. Age-specific incidence rates for norovirus-associated infectious intestinal disease

Age Group	Cases, No	Person-Years	Cases/1000 Person-Years, No (95% CI)
< 1 y	5	26.9	**178.2** (70.5-450.0)
1-5 y	26	190.8	**137.3** (92.6-203.4)
5-15 y	26	424.1	**59.6** (36.8-96.5)
15-64 y	103	2647.8	**39.0** (31.3-48.7)
> 65 y	38	1369.1	**27.7** (19.6-39.1)

Reproduction from O'Brian *et al*. J Infect Dis 2016 [124].

Surveillance

The incidence varies between years as a result of different GII.4 norovirus variants entering the population. Since the mid-1990th we have been used to have a high incidence year about every second or third year, with a peak during 2002 in Sweden and elsewhere [125, 126]. But, the last high incidence year in Sweden was winter season 2012.

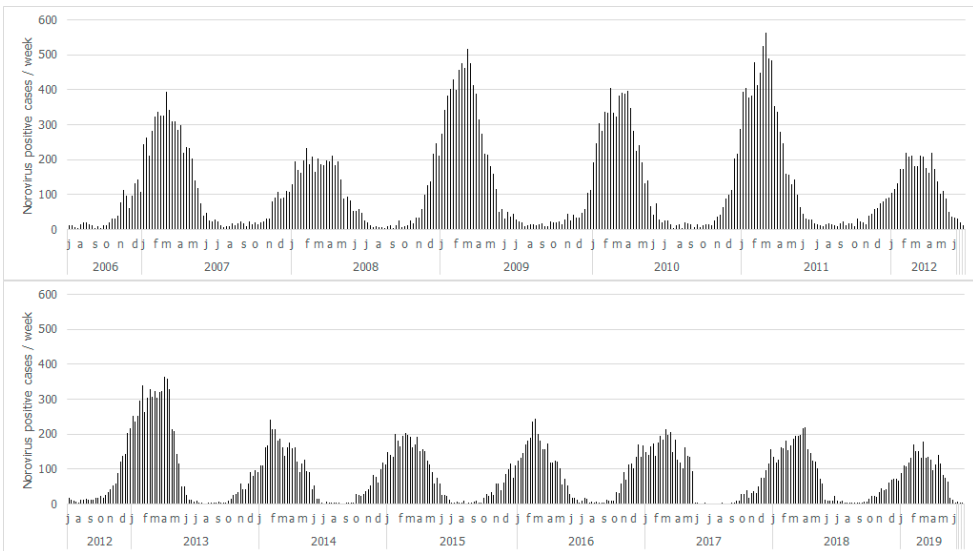

Figure 4. Laboratory surveillance of norovirus in Sweden, norovirus positve results per week. Data provided by the Public Health Agency of Sweden (Folkhälsomyndigheten).

In Sweden national surveillance in based on two data inputs. Firstly, the voluntary laboratory reporting, which is collected and published weekly during the winter season by the Public Health Agency of Sweden (Figure 4). This is based on the norovirus results of nearly all laboratories in Sweden. The majority of norovirus samples sent for analysis are from hospitalised patients. Secondly, syndromic surveillance based on analysis of web searches for "vomiting" at the health information website "1177-Vårdguiden" (Figure 5). The peak or slope of the syndromic surveillance precedes the laboratory surveillance with 2-3 weeks and can thus help healthcare to anticipate an increased norovirus activity [127].

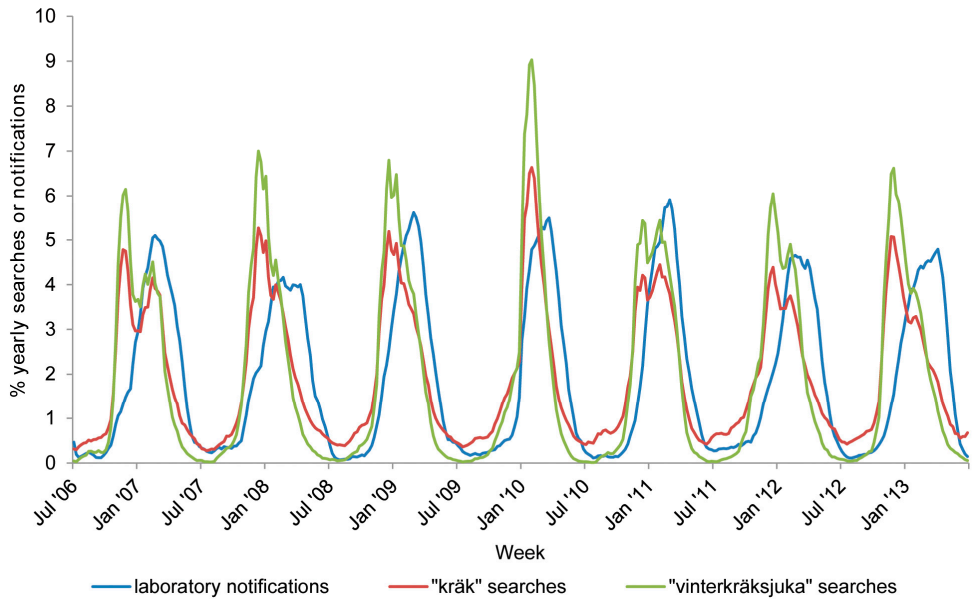

Figure 5. Webb searches for vomiting, winter vomiting disease together with laboratory notification of norovirus to Swedsh Public Agency. Reprint with permission from Edelstein et al [127].

Surveillance of genotype distribution is no longer in operation in Sweden but information of circling genotypes and variants is available from Public Health England and International collaborations (NoroNet) [128]. Global yearly proportion of different GII.4 variants is presented in Figure 6.

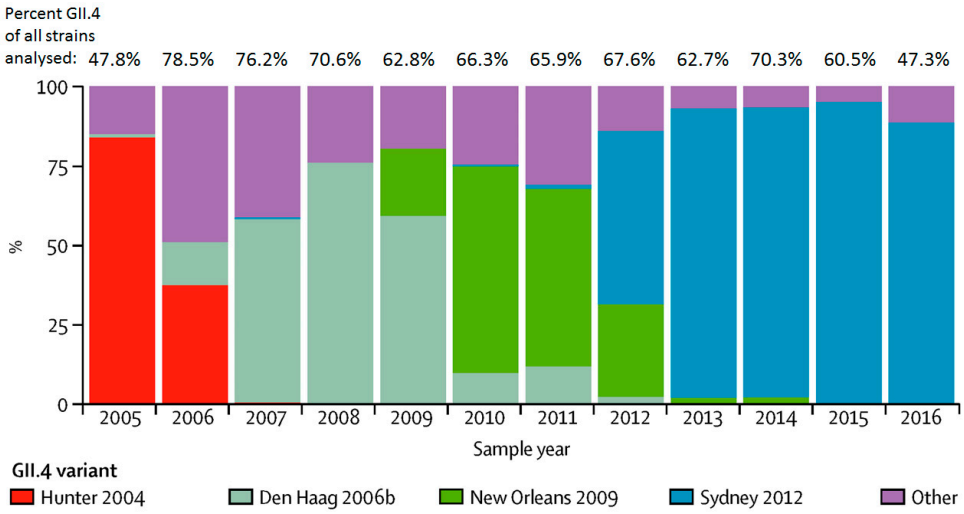

Figure 6. Proportion of GII.4 of all genotypes sequenced and proportion of different GII.4 variants per year. Reprint with permission of van Beek et al . Lancet Inf Dis 2018. © 2018Elsevier HSJournals.

Seasonality

Norovirus infections occur all around the year but in the temperate northern hemisphere norovirus activity increases markedly during the winter months (December-February) [129, 130]. In Sweden the season often peaks in February-Mars and last until early May. In the southern hemisphere activity peak in June to August. Climatic factors seem to play an important role in this dynamics, but the exact genesis is still obscure. An association between norovirus activity and cold and dry climate has been observed [131]. This, in the same context as influenza virus, is hypothesised to be explained by the higher survival rate of certain viruses in low absolute or relative humidity [132].

> "Each time when cholera has been introduced into England in the autumn, it has made but little progress, and has lingered rather than flourished during the winter and spring, to increase gradually during the following summer, reach its climax at the latter part of summer, and decline somewhat rapidly as the cool days of autumn set in."
>
> John Snow, "On the mode of communication of cholera", 1855

Transmission

The norovirus is excreted in faecal matter and vomitus and from there it must reach the gastrointestinal tract of another susceptible individual to complete transmission, by the faecal-oral route. Noroviruses have many features that optimize transmission, summarized in Table 4.

Table 4. Summary of factors facilitating norovius transmission.

Factor	Evidence	Reference
Large human reservoir, asymptomatic carriage and prolonged shedding	Asymptomatic carriage is common in children. Shedding is common 2-3 weeks after infection, longer in immunocompromised, elderly and infants.	[70, 99, 105, 106, 120, 133]
Shedding in high concentrations	The concentration in diarrhoeal samples are approximately 10x 10^{10} virus copies per gram faeces and 10x10^{5} virus copies per millilitre emesis.	[98-100]
Widespread dissemination by vomits	Simulated vomiting events have shown that a single vomiting event can contaminate a large area.	[100, 134, 135]
Possible aerosolization	Aerosol production and possible airborne dispersal or direct transmission by inhalation	[136, 137]
Low infecting dose	Estimated to 18-1000 virus particles.	[60, 61]
Environmental stability	Noroviruses may remain infectious for 2 week or longer on surfaces and 2 months in water.	[138-140]
Resistance to disinfection	Noroviruses are resistant to alcohols and relatively resistant to other means of deactivation.	[141-143]
Limited immunity and high diversity	Immunity limited and varied, estimated to 2-8 years. 30 genotypes and additional variant can infect humans.	[23, 46, 53]

Food- and waterborne transmission

Norovirus can use water or food products as vehicles and cause disease when ingested [144]. These routes of transmission are rare in health care-settings, but contamination during production or preparation of food products is always a risk [145-147].

Direct and indirect contact transmission

The virus can also contaminate the environment by dissemination from diarrhoeal or vomiting events, or by the hands of the infected individual. Touching these contaminated surfaces, or faecal matter or vomitus directly, with hands that later touch food or are inserted in the mouth also complete transmission [148]. The dispersal of viruses from vomiting events is enhanced by the often projectile nature of the vomiting associated with norovirus gastroenteritis. Large droplets can contaminate the area a few meters away from the vomiting event [134, 135].

Droplet and airborne transmission

Vomiting events produce a large amount of droplets of different size. If large droplets reach the mouth of another individual, direct droplet transmission occurs.

During vomiting, also smaller droplets are produced, which may evaporate to even smaller droplet nuclei. These droplet nuclei have a low settling velocity and can stay airborne for long periods and move with indoor air currents [149-151]. Simulated vomiting have been shown to produce aerosols with virus containing droplet nuclei [152]. Norovirus containing droplet nuclei would have the potential to contaminate areas further away from the infected individual [136]. Direct airborne transmission, by inhalation of norovirus containing aerosol, deposition of the virus in the upper respiratory tract and thereafter swallowed, has also been proposed [153]. Nenonen *et al.* detected norovirus RNA in hospital airborne dust and Bonifait *et al.* detected norovirus RNA in hospital air during outbreaks [136, 137]. The implications of this possible transmission route are not fully comprehended, but airborne transmission has been considered the most likely pathway in a number of reported outbreaks [154-159].

Becoming infected after meeting another ill individual, is often called person-to-person transmission, irrespective if transmission was by any direct or indirect route. When exposure to a potentially contaminated environment is suspected and foodborne, waterborne and person-to-person transmission are excluded, this is referred to as environmental transmission. In other cases the transmission mode is unknown (Figure 7).

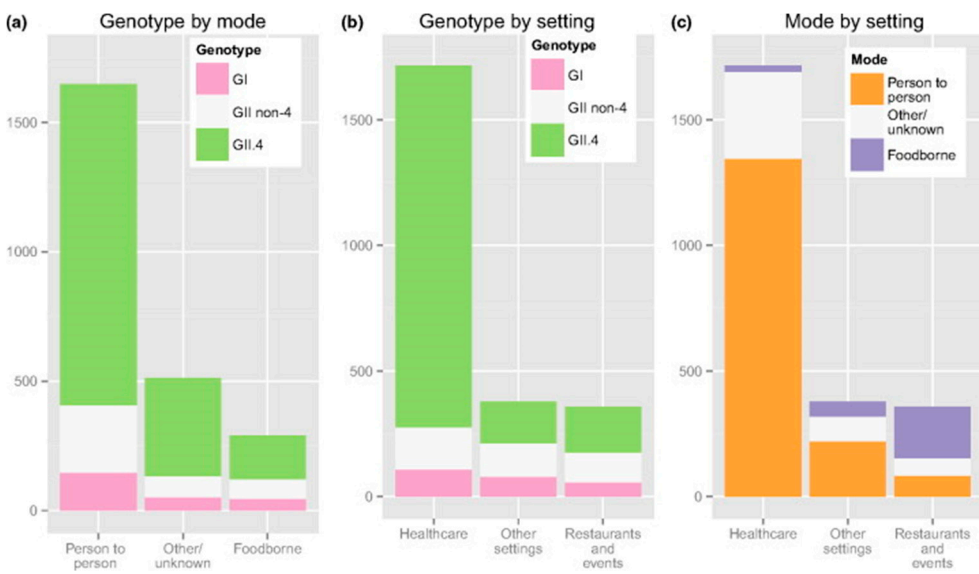

Figure 7. Distrubution of genotypes by mode of transmission (a), outbreak setting (b) and mode of transmission (c) from 2895 norovirus outbreaks reported to CaliciNet 2009-2013. Adapted from Vega et al [160]. Reprint with permission from Barclay *et al.* Clin Micro Infect 2014 [147]. ©2014 European Society of Clinical Microbiology and Infectious Diseases.

Outbreaks

Noroviruses are infamous and sometime feared for their high transmissibility and outbreak potential. Outbreaks are described in a number of different settings including cruise ships [161], airplanes [162], restaurants [157, 163] , schools and children day care centres [3, 69, 158], concert halls [164], hotels [165, 166], military [167], football matches [168, 169], and wherever people meet, or eat.

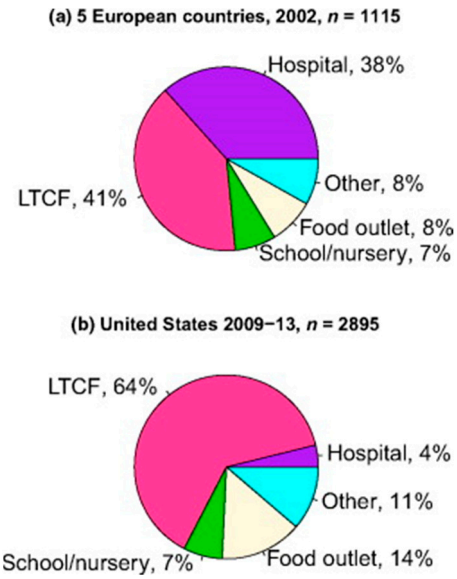

Figure 8. Setting of (a) norovirus outbreaks reported in five European countries with broad-based surveillance, 2002, n = 1115, and (b) the USA, 2009–2013, n = 2895. Long Term Care Facility (LTCF). Adapted from Lopman et al.[14] and Vega et al[160]. Reprint with permision of Barclay *et al.* Clin Micro Infect 2014 [147]. ©2014 European Society of Clinical Microbiology and Infectious Diseases.

On a society scale reported norovirus outbreaks are most frequent in healthcare facilities. In Figure 8, the distributions of reported outbreaks in Europe and USA are shown. The cause for the differences between the regions is yet to be elucidated but might be due to reporting bias.

> "The most terrible outbreak of cholera which ever occurred in this kingdom, is probably that which took place in Broad Street, Golden Square, and the adjoining streets, a few weeks ago. Within two hundred and fifty yards of the spot where Cambridge Street joins Broad Street, there were upwards of five hundred fatal attacks of cholera in ten days. The mortality in this limited area probably equals any that was ever caused in this country, even by the plague; and it was much more sudden, as the greater number of cases terminated in a few hours".
>
> John Snow, "On the mode of communication of cholera", 1855.

Norovirus genotypes in outbreaks

The different genotypes seem to have found special niches where they are more adapted to transmit. Genogroup I is more prevalent in waterborne outbreaks and is also overrepresented in foodborne outbreaks [96, 170, 171]. A wider spectrum of genotypes is seen in community outbreaks, especially those affecting children, where multiple genotypes circulate and herd-immunity might be less established [114]. The norovirus GII.4 strains dominate in healthcare settings, accounting for approximately 85 % of all hospital outbreaks. [172]. In Figure 9 the distribution of genotypes in different, mostly outbreak, settings is presented.

An outbreak is defined by cases being connected in time and space, sharing a common exposure. In clusters, on the other hand, information about exposure is lacking. Outbreaks can be investigated with two different methods. Either epidemiological methods, studying the relationship between the cases.

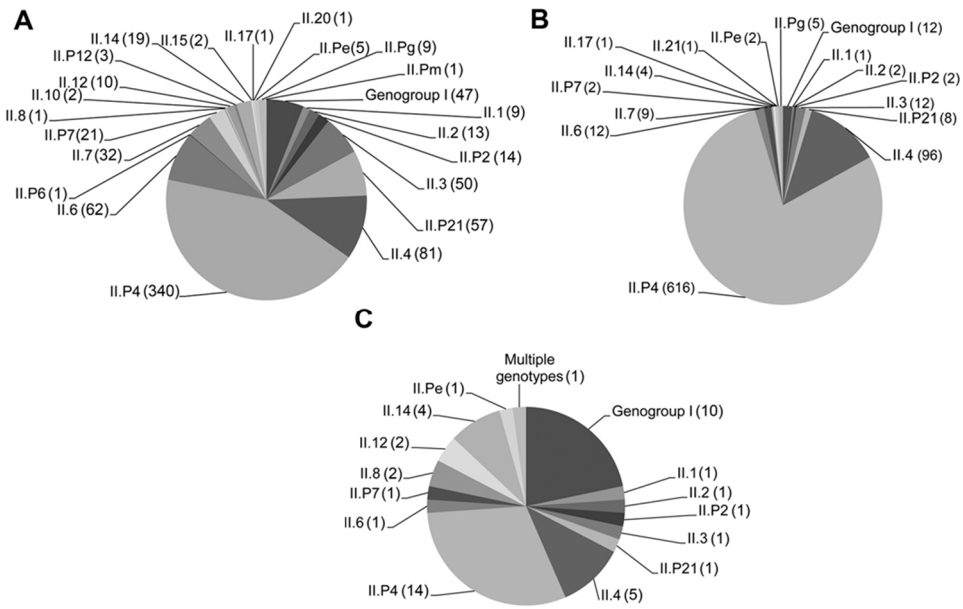

Figure 9. Distribution of norovirus genotypes of isolates from stool samples of A) patients in community settings (n = 781 samples), B) patients in health care settings (n = 785 samples), and C) patients in foodborne outbreaks (n = 46 samples), Denmark, 2006–2010From each clinic and hospital ward, only the first sample with an assigned genotype per calendar month is included. Values in parentheses are numbers of isolates with a specific genotype or genogroup. With permission from Franck et al, Emerg Infect Dis 2014 [114].

Note: Genotypes marked with a P are based on the polymerase gene sequence. GII.P4 corresdonds fair to GII.4, (from the capsid gene).

Another method is the genetic examination of the different virus strains involved in a possible outbreak scenario. An analysis of the relationship in a phylogenetic tree, may suggest a connection between cases. By combining detailed viral and epidemiological data, sometimes called molecular epidemiology, it is possible to back-track when and where transmission possible took place [173]. In some cases, a confined epidemiological outbreak is constituted by multiple virus strains or multiple epidemiological outbreaks are caused by a single strain [174-176].

Outbreak surveillance

Surveillance is essential for providing information for prevention and control of norovirus outbreaks. An optimal surveillance system is simple in design, flexible and stable, with representative high data quality, high acceptability of participants, a good sensitivity and predictive value, gets results timely enough for action, preferably also useful and involving low cost and workload [177, 178]. Surveillance systems can be active, with an active search for outbreaks, or passive, relying on reporting or laboratory results.

Sweden do not use a national surveillance system for healthcare associated norovirus outbreaks, as opposed to many other countries. Unites States of America has three intertwined outbreaks surveillance systems; NoroSTAT where suspected outbreaks are notified, NORS where epidemiological investigations are collected and CaliciNet where data on norovirus strains are uploaded [179]. England and Germany also have Web-based surveillance systems of hospital norovirus outbreaks [180-183].

Waterborne outbreaks

Outbreaks caused by norovirus contaminated drinking water are usually large and often with a sudden start in a confined geographic area. Kaplan *et al.* were the first to report a norovirus outbreak associated with water supply, affecting 1500 individuals [184]. Many similar outbreaks have since been reported, also in Sweden [185, 186]. Waterborne outbreaks are not always associated with drinking water but may be linked to different water activity [187, 188].

> "On proceeding to the spot, I found that nearly all the deaths had taken place within a short distance of the pump."
>
> John Snow, "On the mode of communication of cholera", 1855

Foodborne outbreaks

Food can transmit norovirus by contamination at production site or during preparation. Oysters are the classical vehicle of foodborne norovirus, that bridge between water and foodborne transmission, as they are thought to concentrate viruses when filtering water. The oyster-norovirus association has led to a real

industrial concern [189-191]. Also lettuce and raspberries are often implicated [192-195]. It has been suggested that these food products might have or gain a binding affinity to the virus, in addition to sometimes being irrigated with contaminated water [196]. There are also multiple outbreak reports implicating food handlers or chefs with symptomatic or asymptomatic norovirus infection as the source [197, 198].

> "It is not unlikely that some of the cases of cholera which spring up without any apparent connection with previous cases, may be communicated through articles of diet. It is the practice of the poor people, who gain a living by selling fruit and other articles in the streets, to keep their stock in very crowded rooms in which they live... I often saw baskets of fruit pushed under the beds of sick patients, in close proximity with the chamber utensils."
>
> John Snow, "On the mode of communication of cholera", 1855

Outbreaks in healthcare facilities

The majority of all reported norovirus outbreaks occur in healthcare settings. Both hospitals and nursing homes are affected [147, 160, 199-201]. Norovirus accounts for approximately 65% - 90% of all gastrointestinal outbreaks in hospitals [181, 202, 203].

Burden of hospital outbreaks

The outbreaks cause excess morbidity, prolonged hospital stay and additional mortality among vulnerable inpatients. The healthcare facilities suffer from infected healthcare workers, economic loss and organisational disruption. Hospital outbreaks often result in a reduction of available beds, blocking new admissions [204-206].

In a study 2002-2003 in Avon, England, Lopman *et al.* calculated that the cost of nosocomial gastroenteritis outbreaks was 400.000 £ (≈ 4.800.000 SEK) per 1000 beds, including costs for empty beds and ill staff [203]. Danial *et al*, conducted a similar study in Scotland 2008-2009 and reached a mean cost of approximately 261.000 £ (3.100.000 SEK) per 1000 beds/season [207]. A more recent analysis estimated the total direct cost of norovirus infections for the healthcare system in England to 108 million £ (1.300 million SEK), and another 298 million £ in opportunity loss of 6300 lost quality-adjusted life-years [206].

Control of hospital outbreaks

When an outbreak at a ward is established multiple measures are recommended to limit and control the outbreak as fast as possible. If control fails, hospital outbreaks may ultimately involve hundreds of patients and last for months with high total costs [208, 209]. The evidence backing these control measures is unfortunately week. In

the guidelines from CDC/HICPAC 2011, the British Health Protection Agency 2012, and the Public Health Agency of Sweden 2014, few of the recommendations are supported by even low-quality evidence [210-212]. In fact, the attempts of providing evidence have shown no clear effect of control measures [213, 214]. Still, all the international guidelines recommend similar and sound infection control principals to contain norovirus outbreaks, including promotion of hand hygiene, patient isolation and cohorting, exclusion and quarantine of ill staff, cleaning and disinfection, visitor restrictions and ultimately ward closure [147].

Ward closure

The most controversial of the recommendations is ward closure, as this measure have a high economic cost and may contribute to the shortage of beds [215, 216]. Lopman *et al.* reported 2004 that if a unit was rapidly closed (within 3 days) outbreaks were contained faster, as the mean outbreak duration was only 7.9 days in this group versus 15.4 days in the units that were not closed or closed later [203]. The analysis have been repeated in another larger cohort of outbreaks with similar results – *but* with the additional remark that situations differed between wards that closed and not closed within three days and, wards that did not close at all had a similar outbreak duration as wards with prompt closure, thus making the interpretation difficult [217]. Ward closure might still limit additional outbreaks and are modelled to be cost effective in certain situations [215]. Because of the high cost and lack of evidence many hospitals try to reduce ward closure in favour of cohorting of patients.

Isolation and cohorting of patients

Patients with norovirus infections are normally admitted to single occupancy rooms with an adjacent toilet. Healthcare workers use contact precautions, including gloves and aprons to prevent personal contamination. Face masks are used if there is a risk of droplets from vomiting or splash [210-212]. This isolation is kept during the symptomatic period and usually for at least an extra 48 hours, just in case. Cohorting of patients refers to the separation of symptomatic patients from unexposed asymptomatic patients. Since the attack rate is high in patients exposed of norovirus in a room, symptomatic and exposed patients are collectively often managed as a single cohort [70]. The staff is also separated into these cohorts to minimize transfer between the compartments. This compartmentalization has been evaluated and found to result in less bed-days lost as more beds are available for new admissions, but no effect on the number of patients or staff affected per outbreaks compared to ward closure [218, 219]. Compartmentalization of the ward requires additional staff resources and can thus sometimes be difficult to establish.

> "When a case of cholera or other communicable disease appears among persons living in a crowded room, the healthy should be removed to another apartment, where it is practicable, leaving only those who are useful to wait on the sick."
>
> John Snow, "On the mode of communication of cholera", 1855

Hand hygiene

Hands are the most important vehicle for pathogen transfer in healthcare [220]. That is probably the case also for norovirus. Norovirus genomes are prevalent on the hands of patients and healthcare workers during outbreaks [221]. Inactivation or removal of the virus from the hands is essential to reduce transmission, but the regularly used alcohols only have a limited effect as a virucidal agent [141]. How limited has been difficult to establish since most of the experiments have been forced to use surrogates for human norovirus that might behave differently from the real study object. Recently, as an early result of the human norovirus culture breakthrough, ethanol was shown to only slightly reduce, but not completely inactivate noroviruses [142]. Instead rubbing of the hands in water rinse with soap is recommended [147, 210-212]. This method has been found effective for reducing norovirus contamination of hands [222].

> "The strictest cleanliness should be observed by those about the sick. There should be a hand-basin, water, and towel, in every room where there is a cholera patient, and care should be taken that they are frequently used by the nurse and other attendants, more particularly before touching any food."
>
> John Snow, "On the mode of communication of cholera", 1855

Cleaning and disinfection

Noroviruses are stable in the environment and resistant to different disinfection agents. Norovirus are able to stay viable and transmit disease from various surfaces for days or weeks [138, 162, 223]. Genetic material of noroviruses have been found on multiple surfaces in the hospital environment, including soap dispensers, computer keyboards, blood pressure machines, door handles, during and after outbreaks even after cleaning [136, 224, 225]. Transmission from these surfaces are believed to play a role in outbreak persistence [226, 227]. Cleaning has been shown to reduce the level of contamination [224, 228]. Enhanced cleaning has also been perceived to be important in reducing and controlling outbreaks, but the interpretation is difficult due to the varied norovirus epidemiology between years [229, 230].

Bundles

The prevention and control of norovirus outbreaks are likely to be multidimensional. In the reported studies of successful use of a bundle approach, all of the above aspects have been included with the addition of visitor regulations, staff policy, education, process measures and audit, and surveillance [230, 231].

Aims of the present investigation

To conclude, norovirus is very contagious, outbreaks in hospitals are frequent, disruptive and difficult to control. The preferred action would be to prevent transmission and halt outbreak development early.

The overall aim of this thesis was to investigate how norovirus outbreaks in hospitals can be more effectively prevented by a better understanding of the mode of transmission and factors related to outbreak emergence and surveillance.

The specific aims were:

- To estimate the norovirus outbreak incidence in hospitals in southern Sweden.
- To analyse different surveillance systems of norovirus outbreaks in hospitals.
- To investigate factors associated with emergence of norovirus outbreaks in hospitals.
- To investigate possible airborne transmission by examining the source, concentration, size of norovirus carrying particles and potential relevance of airborne norovirus in hospitals.
- To investigate norovirus environmental contamination as a mode of transmission by analysing the risk of acquiring norovirus infection after admission to a room previously occupied by a norovirus infected patient.
- To investigate the risk of sharing room with a patient with newly resolved norovirus infection.

Patients and methods

Setting

The data included in this thesis was collected from hospitals in Region Skåne, southern Sweden between 2010 and 2018.

Hospitals

Region Skåne, the southernmost region in Sweden, organizes all healthcare for its 1.2 million inhabitants. Ten hospitals serve the inhabitants with hospital care. Two, the jointly organized Lund and Malmö university hospitals, include tertiary care levels. Two additional hospitals, in Helsingborg and Kristianstad, are also referral hospitals. The other five medium to small sized hospitals, still include emergency departments. One hospital, the smallest in Skåne with just two wards, is privately run.

In 2012, the total 194 wards in Skåne, including psychiatric care, had a medium of 16 beds (IQR 11-22) per ward. Room size varied in size between single rooms to maximum four-bed rooms.

Infectious Disease wards

The four largest hospitals have separate Infectious Disease wards, where patients with complicated infections or infectious diseases requiring isolation precautions, such as norovirus gastroenteritis, are primarily admitted. The rooms at the Infectious Disease wards, many used alternately as single or double rooms, have ante-rooms and (in most cases) specially designed ventilation system to minimize transmission between rooms. In total there are five Infectious Disease wards in Region Skåne, all with a similar case mix, except for one of the wards in Malmö hospital, which includes a five bed intensive care unit.

Infection Control team

One single Infection Control team (ICT), consisting of about 16-20 infections control nurses and four infection control doctors, serve all hospitals in Region Skåne. The ICT issues regional guidelines regarding isolation precautions and cleaning instructions for specific contagious diseases, including norovirus infection.

Clinical Microbiology department

Region Skåne is served by a single clinical microbiological laboratory, where diagnostics of all gastrointestinal pathogens, including norovirus, are performed. The norovirus diagnostic method use RT-PCT for detection of genogroup I and II, respectively [111] and is run once a day during weekdays and occasionally on holidays.

Study design

The four research papers included in this thesis use different designs and methods and are essentially based on three different data sets. In Figure 10 an overview of the data sets, including study periods, patients and outbreaks and study design used, is presented.

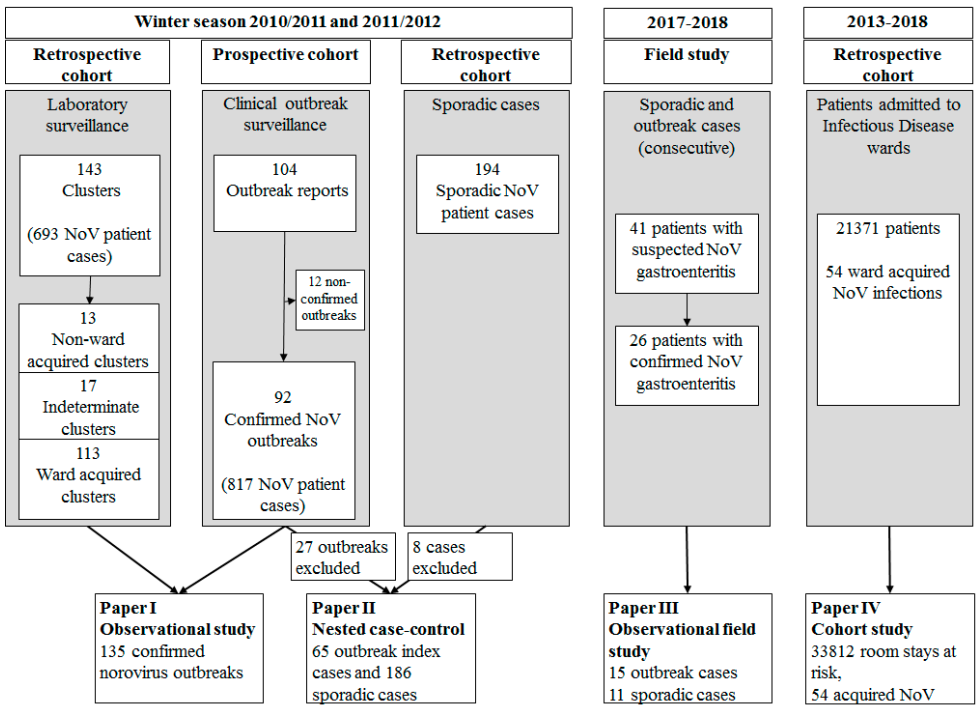

Figure 10. Patients and outbreaks included in the four research papers, with study periods, collection method and study design.

Paper I

All the hospital wards in Region Skåne constituted the cohort. An observational surveillance study was performed, comparing norovirus outbreaks detected by a clinical surveillance system based on reporting to the Infection Control team and ward-acquired norovirus clusters detected by laboratory surveillance of norovirus tests combined with admission data. The incidence of norovirus outbreaks in hospitals in a large healthcare region was calculated.

Paper II

The data on outbreaks and the clusters found in Paper I was used to identify the first individual in each outbreak, the index case (or more correctly the primary case). These were compared in a nested case-control study with sporadic cases to identify specific risk factors for outbreak development related to symptoms, room-size, patient and viral characteristics.

Paper III

A prospective observational field study was performed. Air was repeatedly collected from the proximity of patients with symptomatic norovirus infection. The association between a norovirus positive air samples and time since the last vomiting or diarrhoeal event was analysed. The result of the air samples was also described in the epidemic context, whether related to an outbreak or not. The concentration of norovirus RNA in the air was also estimated and the size of norovirus carrying particles was measured.

Paper IV

A cohort of all patients admitted to any of the five Infectious Disease wards in Skåne 2013-2018 was analysed. Exposure by admission to a room with a prior occupant with norovirus was analysed for association to acquisition of norovirus infection, compared with unexposed. A secondary exposure, risk of sharing a room with a patient with resolved norovirus infection, was also explored.

Data sources and collection

The data used in this thesis is derived from four electronic data sources; medical records, patient administrative database, room admission data base, microbiological database and from prospectively collected outbreak and field study data and samples.

Medical records

In Region Skåne all, but the small private hospital, use Melior (Cerner®), as an electronic medical record system. In Melior, patient data are formatted as text and transcribed by the nurse and physician. Information about notable symptoms of gastroenteritis is documented in the medical records in the absolute majority of cases. A special section of the patient medical record concerning basic body functions, including bowel movements, is regularly used and documented by the assistant nurse.

Medical records were used in a small validation part in Paper I, in order to estimate the delay from symptom onset to norovirus sampling at the wards. In Paper II and IV, medical records were the main data source for information about symptom onset and in Paper II also clinical manifestations of norovirus infection and patient characteristics. In Paper III, medical records were occasionally used as complement to other primary data sources.

Patient administrative database

To document admission and discharge from the hospital and wards, PASIS (Tieto®) is used by the administrative staff of the wards. The database is also used for economy and hospital monitoring purposes.

The patient administrative database was used in Paper I and II as the data source for date of ward admission and discharge of norovirus positive patients.

Room admission database

The room admission database is in essence a part of Melior, where patients, wards, room numbers and time of room admission and discharge are searchable. This database was operational starting from May 2013 and October 2013 in different hospitals. Before this database was installed, three different systems in different hospitals could trace patients to specific rooms. The patient's admission time to the assigned room is documented by the ward staff.

The new room admission database was used as a primary data source in Paper IV, and occasionally used as a complementary data source in Paper III. The old databases of room assignment were used in Paper II.

Microbiological database

All results of clinical microbiological testing are stored in the microbiologic database WWBakt (Autonik®) used in Region Skåne. Sampling ward, sampling time, time of reception at the laboratory, time of final result and the clinical reason for ordering the test are all documented.

The microbiological database was used in paper I-IV. All the norovirus samples analysed in paper I, II and IV were ordered at the discretion of the clinicians, with no attempt to increase testing during the studies. In Paper III, a confirmatory test of norovirus infection was sometimes requested by the investigators.

Outbreak data

In order to study the characteristics of norovirus outbreaks in hospitals, a protocol for outbreak documentation was developed. Data included specific information about the first four patients and staff of the outbreak, control measures taken and a summary of the total outbreak and an epidemic curve. The data, documented by the Infection control team, was collected by daily contact with the staff of the afflicted wards. The outbreak data was used in Paper I and for identifying index cases in Paper II.

Field study data

In Paper III, information about time of last vomiting diarrhoea, onset of symptoms and room size was collected directly from the patients, when possible, or otherwise from the medical staff of the ward.

Air collection

In Paper III air was collected as original data. A Coriolis µ (Bertin Instruments®) air sampler cyclone was used for the primary study objective, operating at 200 L/min for 10 minutes at each sample collection. Next generation Impactor (Copley Scientific®) was used for particle size analysis. The impactor operated at 17 L/min for 17-120 hours. Both air samplers are shown in Figure 11.

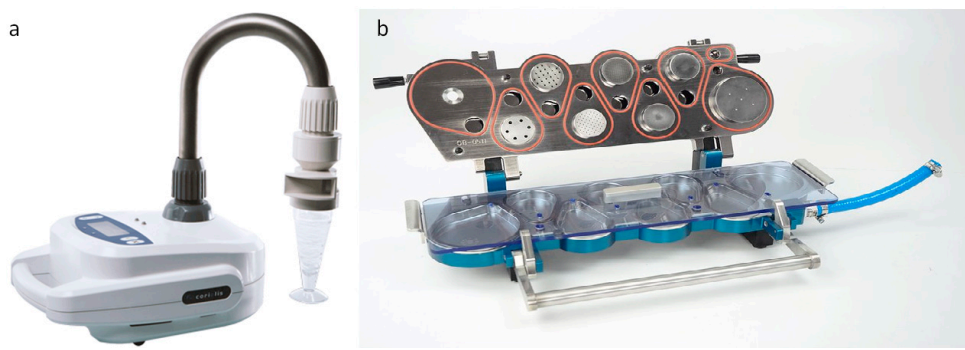

Fig 11. Air sampler used in Paper III, a) Coriolis air cyclone (Bertin Instruments®), b) Next generation impactor (Copley Scientific®). With permission.

Definitions

Case

A suspected case of norovirus infection was defined as an individual with ≥ 2 episodes of diarrhoea or vomiting within 24 hours that could not be attributed to any other illness or medication. A confirmed case of norovirus infection was a suspected case with a positive norovirus test.

In Paper IV patients with positive norovirus test sampled five days before admission to three days after discharge was regarded as a current norovirus infection.

Mode of acquisition

Onset of disease ≥ 48 hours after admission to the ward was defined as a ward acquired norovirus infection. Onset ≤ 48 hours before or since admission was defined as community acquired in Paper IV. In Paper II this period was split into "non-ward acquired infections" ≤ 24 hours and indeterminate if 24 - 48 hours.

In Paper I definitions were based on time from admission to a hospital/ward to sampling; community/non-ward acquired 0-1 days, indeterminate 2-4 days and nosocomial/ward acquired > 4 days.

Outbreak

In this thesis an outbreaks is always referred to a ward, taking no account if the ward outbreak was a part of a larger hospital outbreak.

Both in Paper I and Paper II, a norovirus outbreak was defined as two or more cases with onset within five days of each other, of which at least one case was suspected of clinical transmission within the ward. The outbreaks was set to end after a period of seven days after the last symptom.

In Paper III an outbreak was defined as the occurrence of two or more patients with confirmed norovirus infections simultaneously at the ward, including at least one patient with a probable ward-acquired infection.

Cluster

In Paper I, a cluster definition was used. A cluster is a surveillance term used when no exposure data is available. The cluster definition used was two patients with positive norovirus tests taken within five days at a ward. The cluster ended after nine days without no new positive tests at the ward. The clusters were categorized into three groups depending on the mode of acquisition of the included patients. Ward-acquired clusters, with at least one patient admitted minimum five days before sampling, were set to be equivalent to an outbreak.

Prior room occupants and roommates

In Paper IV, a prior room occupant with norovirus infection was defined as a room occupied by a patients with norovirus infection the past seven days. To have a roommate with norovirus infection was defined into two groups; 1) roommates with ongoing norovirus infection with symptoms of gastroenteritis within < 48 hours and 2) roommates with recent norovirus infection with symptoms resolved since ≥ 48 hours.

Colonization pressure

In paper IV, a variable representing the colonisation pressure of norovirus infections at the ward, exposing patients to a possible risk of norovirus acquisition, was used, similar to previously described [232]. The colonization pressure was defined as the cumulative proportion patients with norovirus infection at the ward during room stay until room discharge or onset of norovirus infection. A colonization pressure > 0% represents minimum three hours spent at the ward together with at least one norovirus patient and a 10 % increase represents one additional day with two norovirus infected patients at the same 20 bed ward.

Analysis

Laboratory analysis

All analyses were performed at the Department of Clinical Microbiology, Lund. Routine method for norovirus diagnosis was used in all Papers [111]. Sequencing for genotyping and variant determination was used in Paper II and III [114]. In Paper III samples were concentrated, using centrifugal filtration (Amicon Ultra-15, Merck Millipore®), and the final Ct-value of the RT-PCR was converted to a specific virus concentration using a standard solution with a known concentration as reference.

Statistical analysis

All statistical analyses were performed using STATA SE (version 14 and 15, StataCorp LLC®). All test were two-tailed and p-values < 0.05 were considered significant. Chi-squared test, t-test and Fischer's exact test were used when appropriate.

Logistic regression

Logistic regression is a statistical method of analysing the log odds of a binary outcome variables, for example outbreak versus no outbreak. In univariable analysis

the effect of one exposure variable is examined. In multivariable analysis many variables are jointly analysed, and the effect of every single variable included is "controlled" for the others.

Clustered data

When planning to analyse grouped data, for example are repeated measures of the same individual or patients treated at one of the included wards, they are not independent of each other. The grouped data may have similarities within the group and the statistical analysis has to take account for this clustering of data. Several ways to do this are described. The method used in this thesis, in Paper III and IV, is called random-effect model. In these models data within a cluster is treated as independent and incorporates both within cluster variation and between cluster variations.

Ethical consideration

The Regional Ethical Review Board in Lund approved the data collection used in this thesis (Dnr 2015/51 and 2016/961). Informed consent was not necessary in any part of these studies, but patients had the right to deny stop air collection at the room if it was found disturbing. Data was anonymised and presented at group level.

Results and discussion

Incidence of norovirus outbreaks in hospitals

To understand the impact of outbreaks and as a basis for planning and decision making, information about incidence is important. Data on incidence is also vital in any attempt to reduce the number of outbreaks by prevention.

In Paper I, incidence of norovirus outbreaks in all 194 hospital wards in Region Skåne was analysed during two consecutive winter seasons (end of November to end of April) 2010-2011 and 2011-2012.

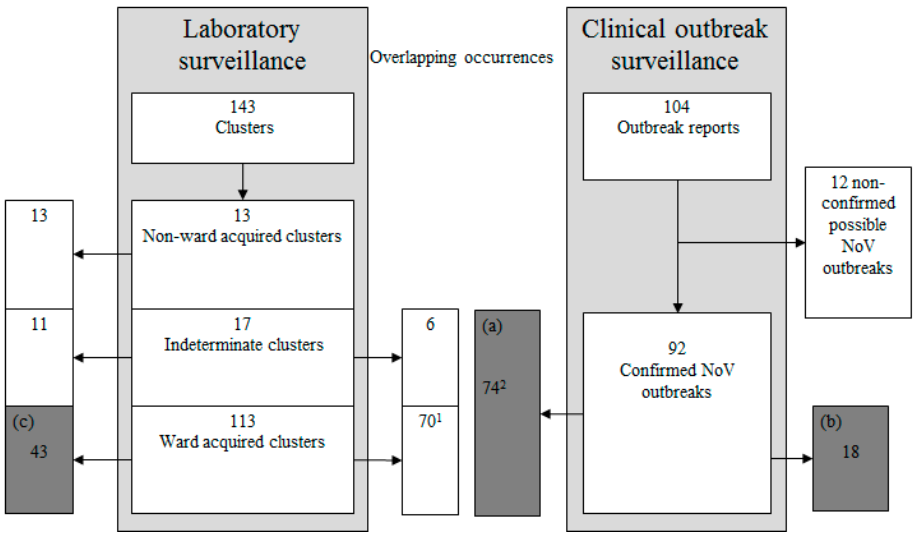

Figure 12. NoV outbreaks marked with dark grey; (a) reported outbreaks with corresponding cluster, identified by both methods, (b) reported outbreaks without corresponding cluster, identified by clinical surveillance only and (c) ward acquired cluster without corresponding clinical outbreak report (non-reported outbreaks), identified with laboratory surveillance only.
[1] One of the 70 ward acquire clusters corresponded to two reported outbreaks.
[2] 69 of the 74 reported outbreaks matched with ward acquired clusters and 5 matched only with indeterminate clusters. 71 of the 74 reported outbreaks matched to a single cluster but three reported outbreaks matched with two clusters.

The clinical surveillance detected 104 outbreaks, of which 92 were confirmed norovirus outbreaks. By using the definition of ward acquired cluster to find possible unreported outbreaks, another 43 non-reported outbreaks were identified. In total 135 norovirus outbreaks were identified (Figure 12). Outbreaks were more common in medical wards ($p < 0.05$) and during the 2010-2011 winter season. The overall incidence was 0.5 outbreaks 2010-2011 and 0.2 outbreaks per ward and season for the two seasons studied (Table 5). During the 2010-2011 and 2011-2012 seasons 43 % and 30 % of all medical wards in Skåne experienced at least one outbreak, respectively.

Table 5. Incidence of confirmed norovirus infections and outbreaks for the winter season 2010-2011 and 2011-2012

Season		2010-2011	2011-2012
Admissions		90800	93700
Acquisitition mode of inpatients with confirmed norovirus infection	Community aqcuired (I[1])	183 (2.0)	91 (1.0)
	Indeterminate (I[1])	159 (1.8)	68 (0.7)
	Nosocomial (I[1])	291 (3.2)	103 (1.1)
	Total (I[1])	633	262
Reported outbreaks / Unreported outbreaks		59 / 33	33 /10
Outbreaks[2] per 1000 admissions		1.0	0.5
Outbreaks[2] per 100 beds		2.8	1.3
Outbreaks[2] per ward		0.5	0.2

[1] Incidence per 1000 admissions

[2] Reported and unreported outbreaks.

Note: Data for all 10 hospitals in Skåne, including medical, surgical pediatric and psyciatric departments, comprising 194 wards and 3300 beds.

The outbreak incidence was similar to previously reports, from which derived data show an incidence of 0.3-0.5 outbreaks per ward-year [203, 207, 233], or and 2.9-7.9 per 100 beds and year [180]. A previous Swedish study, Billgren *et al.* reported that 20 % of all geriatric and medical wards in Stockholm experienced at least one outbreak in 1996 [233]. Increased rate of norovirus outbreaks in larger care units and those with high throughput, and specifically geriatric and medical units, has previously been reported [234]. Units which experienced outbreaks the previous year also seem at increased risk for the following year [233].

It is still difficult to compare incidence rates of different settings and years. For example, the referenced studies have not included psychiatric wards in the analysis.

The incidence observed in paper I is likely an underestimation because: 1) some of the 11 indeterminate clusters, including at least one patients sampled 2-4 days after admission to the ward most represent an outbreak; 2) small outbreaks, with less than four cases, seemed underreported when analysing outbreak size distribution; 3) 8 % of the patients with hospital acquired norovirus infection were not part of any reported outbreak or cluster, indicating undetected or patients not tested for norovirus; 4) A capture-recapture[1] calculation of outbreaks undetected by both surveillance systems generate another 10 outbreaks.

When analysing all norovirus tests over the full two year period, 87 % of all positive tests were sampled during the study periods (unpublished data), inferring that additional outbreaks may have occurred between May and November 2011, outside the study periods.

Surveillance of norovirus outbreaks in hospitals

In Paper I two different surveillance methods were compared. The purpose was to evaluate the present system based on reporting of outbreaks to the Infection Control team and develop a surveillance system based on laboratory data of norovirus test results. The latter surveillance system would have the advantage of being more stable over time and would have the potential to compare outbreak epidemiology of different regions and countries.

Of the 135 confirmed norovirus outbreaks, 74 were detected by both clinical and laboratory surveillance, 18 were identified only by clinical reporting and 43 by laboratory surveillance.

The sensitivity of the clinical surveillance system was 68 %, while the laboratory surveillance reached a sensitivity of 86 %. The positive predictive value of the clinical surveillance was 88 % and higher than the 81 % of the laboratory surveillance (Table 6).

Comments on laboratory surveillance

Laboratory surveillance thus seems to be a simple, stable system with acceptable sensitivity and positive predictive value for detecting outbreaks. The system would preferable be combined with admission data to enable use of the ward acquired cluster definition.

[1] The capture-recapture method can be used for estimates of undetected occurrences by evaluation of the overlap of two incomplete and independent surveillance methods. The less overlap, the more undetected occurrences.

Table 6. Sensitivity and positive predictive value of the two surveillance systems investigated.

Detected by surveillance	New outbreak[1] present		Total	Sensitivity	Positive predictive value
Outbreaks reported by ICT	**Yes**	**No**			
Yes	92	12	104	68 %	88 %
No	43	-			
Cluster by laboratory surveillance[2]	**Yes**	**No**			
Yes	116	27[3]	143	86 %	81 %
No	19[4]	-			
Total	135				

[1] Confirmed reported outbreaks and ward acquired clusters
[2]The laboratory surveillance without admission data.
[3] The 13 non-ward acquired clusters, 11 the indeterminate clusters without a matching outbreak and 3 dublicates clusters.
[4] The 18 outbreaks without corresponding clusters and 1 dublicate outbreak.

The timeliness of the surveillance systems was not compared, but for the laboratory surveillance the time lag from a theoretical clinical outbreak notification was three days. Rapid outbreak response would thus need to act on other signals. Perhaps notification about one single norovirus positive patient would be enough for action, since the probability of a new norovirus positive inpatient to be included in an outbreak was 32 %. This "outbreak risk" could also be used as a quality outcome measure for norovirus outbreak prevention.

The size of outbreaks are difficult to ascertain with the laboratory surveillance system. The mean proportion of norovirus positive patients of all patients cases in reported outbreaks were 56 % (CI 95% 51-61) (unpublished data). In Infectious disease wards the ward acquired clusters included many patients with community acquired infections since admission of new patients continued in spite of seemingly ongoing outbreaks.

Comments on clinical surveillance

Why was the sensitivity of the clinical surveillance by the ICT lower than the laboratory surveillance? This could have many explanations, all associated with the interaction between the wards and the ICT and the perhaps perception of implication and effect of the control measures. A possible tendency that fewer of the outbreaks are reported at the end of the season may be noted. In Figure 13, week of outbreak start and number of ongoing outbreaks are visualised. Some domestic and foreign health services now have Webb-based notification from the ward to the ICT, which might enhance clinical reporting [182].

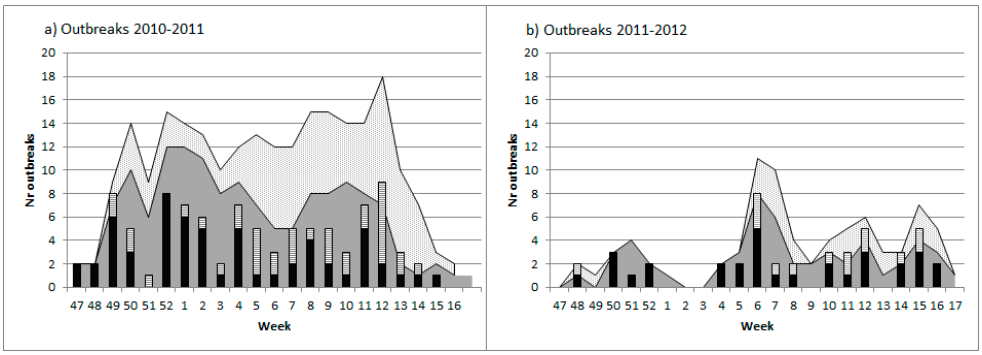

Figure 13. Week of onset of the first case during a) winter season 2010-2011 and b) 2011-2012 in reported outbreask (black bars) and ward acquired clusters(grey bars). Dark grey area represtents ongoing reported outbreaks and light grey area ongoing ward acqurired clusters. (Unpublished data).

Limitations

A perquisite for the laboratory surveillance is that norovirus test are ordered and sampled at the ward. Tests taken, for example, at the emergency department or before ward transfers will not be included in any cluster related to the ward where the patients are presently staying. In this cohort, sampling at the emergency department was rare. Of the 1455 norovirus positive samples analysed during the study periods, 80% were submitted from in-patient wards and 8% and 13% samples were submitted from out-patient hospital departments and primary care, respectively (unpublished data).

In the clinical surveillance, the case definition of all patient and healthcare worker cases included in outbreaks can sometimes be difficult to verify, and the number of included patients in clinical reported outbreaks might be an overestimation of the true number infected. To perform studies with strict definitions based on verifiable data during the often chaotic situation at the outbreaks wards is a challenge.

Definitions of outbreaks and clusters can be argued. The start of an outbreak is often apparent, even if definitions differ somewhat regarding maximum period between cases. When an outbreak or cluster ends is less discussed. We chose a relatively long period to minimize a perceived single outbreak to be classified as two or more.

Practical implications

Laboratory automated surveillance including data admission date, seems to be an adequate method for continuous surveillance of outbreak. Clinical reporting ought to be continued for immediate action and as a complement to the laboratory surveillance.

The norovirus outbreaks

In Paper I, 89 of the 92 outbreaks reported by the ICT included complete data of number of cases. The median number of cases per outbreak was six patient cases (IQR 4-11, max 57) and four healthcare worker cases (IQR 1-9, max 32). Distribution of outbreak size is presented in Figure 14.

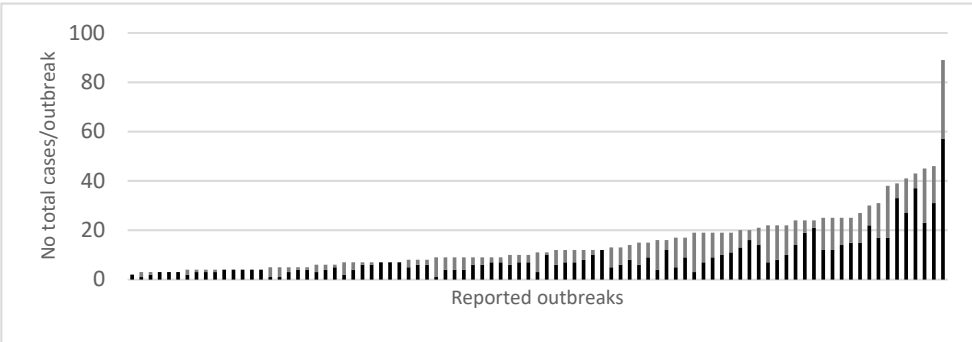

Figure 14. Distribution of outbreak size. Black bars= patient cases, White bars= healthcare worker cases. Unpublished data.

Median outbreak duration was 8 days (IQR 5-12, max 73) from onset of the first to the last case. Ward closure was used as a control measure in 58 % of the reported outbreaks, resulting in approximately 332 total days of ward closure (unpublished data).

Comments on health-care worker cases

The proportion of ill healthcare workers (HCW) was high compared to most other reports, but not all (Table 7). The proportion of ill HCW may be explained by a number of different factors. Hospital ward design is likely of importance. With many single or double occupancy rooms attack rate of patients seem to decrease. In outbreak reports from British hospitals, where the use of larger patient bays is common, a high attack rate among patients often seen, resulting in a high patients / HCW case index. A high HCW / patient ratio at the ward would also result in comparably more HCW contracting norovirus during care. The attack rates among staff is also reported to be higher if the index case is a HCW [235]. Sick-leave reimbursement policy of infections contracted during work might have an impact. Most of the HCW infections reported in Paper I are self-reported illnesses. In Skåne full salary is being paid to HCW during the symptomatic period and until return to work, 24 hours after resolution of symptoms, if nosocomial transmission is

suspected. Inadequate use of personal protective equipment and low adherence to standard and contact precautions is yet another other possible explanation.

When summarizing all reports in Table 7, it is evident the HCWs are at a high risk of contracting norovirus infection during outbreaks, presumably a higher occupational risk than for most other human pathogens.

Table 7. Review patients / healthcare worker distrbution of some published outbreaks in healthcare facilities.

Country	Year	Setting	No. Outbreaks	Patient cases (median)	HCW cases	Patients / HCW	Ref
Sweden	**2010-2012**	**Hospital**	**89**	**817 (6)**	**523 (4)**	**1.5**	**Paper I**
Scotland	2011	Hospital	195	1732	599	2.9	[207]
England	2005-2011	Hospital	135	(14/17)*	(2/2)*	7.5	[219]
England	2007-2010	Hospital	62	636	206	3.1	[218]
Sweden	1996	Hospital / LTCF	54	557	237	2.4	[233]
England	2009-2011	Hospital	2670	(11/12/ 14.5)*	(2/3/2)*	5.5	[217]
Germany	2014-2015	Hospital	7	57	29	2.0	[236]
Germany	2014-2016	Hospital	10	75	39	1.9	[237]
Review	1991-2003	Hospital / LTCF	30	670	363	1.8	[235]
Finland	2006	Hospital	1	240	205	1.2	[208]
USA	2004	Hospital	1	90	245	0.4	[209]

* The study reported separate median value of different time periods.

> "The low rate of mortality amongst medical men and undertakers is worthy of notice. If cholera were propagated by effluvia given off from the patient, or the dead body, as used to be the opinion of those who believed in its communicability; or, if it depended on effluvia lurking about what are by others called infected localities, in either case medical men and undertakers would be peculiarly liable to the disease."
>
> John Snow, "On the mode of communication of cholera", 1855

The start of norovirus outbreaks

In this section some unpublished observations from the investigations of the outbreaks included in Paper I and II are presented in relation to the start of the outbreak.

An examination of the first individual case of the outbreaks, with the earliest observed onset of norovirus gastroenteritis, at the wards of the 91 reported outbreaks was performed. This first individual is often called the index case (the first recorded case in the outbreak), or primary case (the first case in time). In Paper II, and in this thesis I have called this individual the index case, but primary case might be more correct.

In at least 14 outbreaks (data missing in additional three) more than one patient and / or HCW had onset on the same date as the index, but analysis was still performed with the first individual. In six of the 92 outbreaks information was missing or data too incomplete to make a statement about any index case (Table 8).

Table 8. Index cases in the 92 reported outbreaks 2010-2012 (unpublished data).

Unknown	HCW index	Patient index				
		Total	Symptom onset before ward admission	Symptom onset within 24 hours of admission	Symptom onset within 24-48 hours of admission	Symptom onset after 48 hours of admission
6	6	80	21	12	10	37

In six outbreaks the index case was a HCW. This is lower than the previously reported 20 % of the outbreaks having a HCW-index [238, 239]. A patient was the index in 80 of the outbreaks, of which 65 had a positive norovirus test.

In 12 of the 21 index cases with onset before admission, viral gastroenteritis was not suspected at the emergency department, instead the first preliminary diagnosis was, in falling order; chest pain, geriatric fall accidents, syncope / cerebrovascular insult, sepsis and constipation. Patients with undetected norovirus infection have previously been shown to be associated with norovirus epidemiological clusters [240].

Of the 12 index cases with onset within the incubation period (< 24 hours) at least six had family members with gastroenteritis or were transferred from wards or nursing homes with ongoing outbreaks.

Risk factors for norovirus outbreaks

In Paper II the association between outbreak development and specific clinical, ward and virus specific variables in a nested-case control study. The 65 index cases with confirmed norovirus infection were compared with 194 patients with sporadic infection. Several risk factors for outbreak development were found in the multivariable analysis (Table 9).

Table 9. Independent risk factors for outbreak development on multivariable logistic regression

Variable	OR	95 % CL	p-value
Age > 80 y	3.2	1.6 - 6.6	<0.01
Comorbidity	2.3	1.0 - 5.2	0.05
Vomiting	2.6	1.0 - 6.3	0.04
Genogroup II	6.2	1.0 - 39.9	0.06
Days to sampling[1]	1.3	0.9 - 1.8	0.22
Additional patients in the room[2]	1.9	1.3 - 2.6	<0.01
Symptom onset after ward admission[3]	3.5	1.5 - 7.9	<0.01

[1] Days from first symptom at the ward to sampling. OR is for one additional day to sampling from 0 (the first day) to ≥4 days.
[2] Number of patients placed in the same room as the case at the day of symptom onset. OR is for every additional patient in the room from 0 (case treated in single room) to 3 patients.
[3] Symptom onset after ward admission. All onsets after ward admission grouped as one.

Symptom onset and early recognition

Symptom onset after ward admission was strongly associated with outbreak development. Most of the patients with ward admission more than an incubation period (> 48 hours) before the onset presumably contracted the disease at the ward, implying another original source to the outbreak. This source could be an unrecognized symptomatic or asymptomatic patient or HCW, or contaminated environment.

Days from symptom onset to norovirus sampling was used as a surrogate variable for early recognition, which is regarded as a key for outbreak prevention and important for reducing outbreak size [210, 239]. In my analysis this factor did not reach statistical significance. One interpretation of the result is that cases have to be recognized and single room isolation has to be instituted *before* the first symptom at the ward. If recognition is later most of the harm is already done.

Sharing room

Not surprisingly, treating norovirus patients in single occupancy rooms was protective against outbreak development and sharing room with other patients was a risk factor. Single room isolation is a very reasonably strategy when trying to

prevent the spread of a highly infectious virus, and commented as beneficial in multiple observational outbreak reports. But the American CDC guidelines state 2011, that there is only very-low quality evidence to place patients on contact precautions during outbreaks and no evidence for sporadic cases [210]. Harris et al has later provided additional evidence, based on probabilistic reconstruction of chains of transmission, that patients in the same bay as a norovirus patient are at an increased risk of contracting the disease and that proximity is an important factor [241]. In my analysis the risk of an outbreak double with every additional patient within the room.

Elderly patients and comorbidity

Elderly (> 80 years of age) and patients with comorbidity were at increased risk of becoming an index case in an outbreak. These two patient characteristics may serve as a surrogate variable for dependency and care burden and hypothetically more opportunities for transmission by the hands of HCW. Elderly were also more likely to be assigned a multi-occupancy room. Single rooms seemed to be prioritized to younger patients. Age and dependency have previously been implicated as factors of importance for transmission [70, 238].

Vomiting

Vomiting at the ward was experienced by 83 % of the index cases and 73 % % of the sporadic cases, according to medical records. In the multivariable analysis vomiting was associated with outbreak development. Vomiting has been perceived as a transmission enhancer by dissemination of virus particles in the surrounding environment. Chadwick *et al.* was early to report outbreaks probably propagated by vomiting events [155, 156]. Exposure to vomit has been implicated as a risk factor in a review of outbreaks in nursing homes [238], and discussed as a transmission risk in light of evidence from challenge studies [100]. The result of Paper II further strengthens the evidence that transmission may emanate from vomiting events, and the reason for this will be further discussed in Paper III.

Norovirus genotype II.4

As some data on genotype was missing, a secondary analysis was performed including only patients with successfully sequenced infecting virus strains. Genotype GII.4 was associated with outbreak development, but only if vomiting was excluded from the multivariable model. When analysing this further an association between GII.4 and vomiting was noted (OR 2.5; 95% CI: 1.1-5.8; p=0.01). This was interpreted as vomiting was on the pathogenic pathway, used by GII.4, to transmit and cause outbreaks. The reports of GII.4 causing a more severe disease and the GII.4 association to outbreaks in semi-enclosed settings may now

be combined to form a more complete explanatory framework of how virulence, epidemiology and infectivity may be connected.

Limitations

This study has several limitations. The most obvious is the difficulties to classify cases as index and sporadic cases. Firstly, when two or several cases have onset the same date, it is from an epidemiologic perspective very hard to state that one of them was the index (or primary) case that ignited the outbreak. Previous studies of index cases have chosen to classify all cases as index cases in onset were simultaneous, or classify the outbreaks as "several index cases"[238, 239] Similar argument can be held against the inclusion of index cases with ward acquired infection, where the initial transmission remains unknown. Secondly, how is transmission or outbreak associated to the sporadic cases excluded? Two surveillance system were set to secure this, but as has been described in Paper I, undetected outbreaks were with high certainty occurring even with the combined surveillance systems. Thirdly, medical records are imperfect for collection of information about time and characteristics of certain symptoms. Fourthly, information about some important variables, such as staffing of the ward and compliance to standard and contact precautions was lacking.

In spite of the risk of misclassification, of which some likely was non-differential[2], I still believe the result can tell something about factor associated with outbreaks. To exclude categories of index cases also diminish the generalisability of the study.

This is first study to demonstrate risk factors in a study design with analysis of outbreak index cases and sporadic cases not involved in outbreaks, enabling an approximation of the odds ratios of the specific risk factors.

Practical implications

Single room can help prevent norovirus transmission. As a rule, assignment to a single occupancy room at ward admission would be preferable to avoid transmission from unexpected norovirus cases.

[2] Non-differential meaning that misclassification does not bias the result in either way, just dilute the association.

Transmission by air

In Paper III aspects of airborne norovirus was investigated. The aim was to study the prevalence and possible sources to airborne norovirus. The potential for airborne transmission was investigated by measuring virus concentration in air and size of norovirus carrying particles. The possible relevance of airborne norovirus was explored by analysing association to outbreaks.

Forty-one patients with possible norovirus gastroenteritis were identified with ongoing symptomatic disease and multiple air samples were collected. In fifteen of the patients other causes to their gastroenteritis-like illness were subsequently established and norovirus infection was ruled out. In the remaining 26 patients norovirus infection was confirmed, of which 15 were part of ongoing outbreaks and 11 were sporadic cases. In total 86 air samples were collected from the proximity of these patients, in their room, toilet and in the ward corridor. Additional investigation concerning particle size was performed during four outbreaks. Air positive for norovirus RNA was detected in 21 (24 %) of the samples, from 10 (38 %) separate patients.

Source of airborne norovirus

Detection of norovirus positive air was associated with a shorter period of time since the last vomiting ($p<0.01$), even after controlling for diarrhoea. Nine of 14 (64 %) air samples collected within 3 hours from a vomiting episode were norovirus positive. Odds ratio for norovirus detection in an air sample in the room or adjacent toilet within three hours since last vomit was 8.1 (CI 95%: 1.1-64; $p=0.04$) compared to a vomiting event since more than three hours or no vomit at all. The association to time period since last diarrhoea was less clear and not statistically significant (Figure 15).

This data supports the suspicion of vomiting as the major source to norovirus in aerosol. Other sources are certainly possible. Toilet flushing has been shown to produce aerosols [242, 243]. We were not able to ensure how diarrhoeal events were handled and norovirus containing aerosol after toilet flushing could not specifically be investigated in our study. Many patients were immobilized and used diapers, but this was not always recorded. Cleaning with wet mopping has recently been reported to be able to produce norovirus positive aerosols [244]. Cleaning was not recorded as part of the protocol, nor a common event in connection with air sample collection.

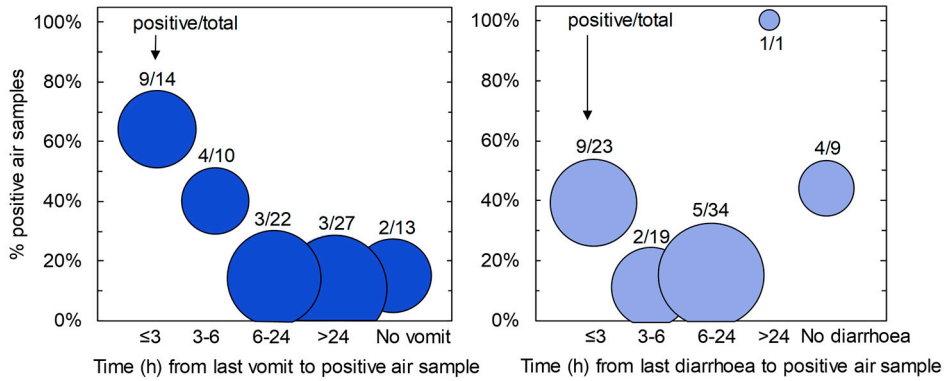

Figure 15. Percent of NoV-positive air samples in relation to time since (A) last vomiting episode and (B) last diarrhea. The area of each bubble is proportional to the total number of air samples within each time interval. The values above each bubble represent the number of positive and total air samples. Abbreviation: NoV, norovirus.

Norovirus concentration in air

Concentration of airborne norovirus RNA ranged between 5-215 norovirus copies/m^3, with a mean of 31 copies/m^3. Bonifait *et al.* reported a slightly higher concentration; range 13.5-2350 copies/m^3 [137]. Concentrations are likely an underestimation of the true concentrations in air, as recovery not is perfect using the liquid cyclone, nor perfect at the Amplicon filtration concentration step before RNA extraction. If the concentration represents viable viruses that reach the upper respiratory tract after inhalation, breathing the air for 10 minutes might hypothetically suffice to cause infection, if calculated with an infectious dose of 18 virus particles and the highest concentration measured. The study by Bonifait *et al.* also found the murine norovirus preserved its infectivity and integrity during aerosolization, implying that might be the case for human norovirus too [137].

The sample with the highest concentration measured (215 copies/m^3) was sampled in a room after a diaper was changed. This individual had multiple measurements with nearly consistent high concentrations, and an outbreak followed shortly at the ward. Also the first samples in this patient's room, before any vomiting or diarrhoeal events had occurred within the room, was positive. Hypothetically, breathing and coughing might cause norovirus containing aerosols as mouthwash samples have been shown to be norovirus positive, especially after vomiting [113]. This single individual supports the hypothesis that some individuals transmit much more efficiently than others and are "superspreader". [245].

Size of norovirus carrying particles

Three of the four outbreak measurements with the impactor sampler yielded positive results. The impactor stages positive for norovirus was different in all measurements; 0.14-0.34 µm, 0.34-0.94 µm and 4.5-> 8.13 µm, respectively. The result implicate that the norovirus found in air are not occasional larger droplets or virus attached to larger dust particles, but real airborne particles, presumably droplet nuclei. These submicrometre particles move freely in air currents and could settle far from their source or be easily inhaled [149, 151]. Indoor relative humidity have a crucial effect on formation of small droplet nuclei. In a low relative humidity indoor environment (which usually is associated to low outside absolute humidity observed during cold, dry weather conditions) evaporation is quick and more and larger droplets may reach the equilibrium size and stay airborne before they hit the floor [149, 246].

Association to outbreaks

Fifteen of the 26 patients were part of 12 separate outbreaks. A positive air sample was collected in the proximity of nine of these patients. When including the impactor air collection, at least one air sample was positive in nine of twelve (75 %) investigated outbreaks, and in seven (58 %) of the ward corridors. Bonifait *et al.* similarly detected norovirus positive air in six of eight (75 %) outbreaks [137]. In our study, norovirus positive air samples were collected from only one of the 11 patients with sporadic infection, and in the case an outbreak emerged a few days later.

This observation should be cautiously interpreted. During outbreaks more patient are ill and thus concentration in corridor air is likely to be higher, though this objection cannot entirely explain the higher prevalence of norovirus positive air within each patient's room. Collecting air close in time from a vomiting event is challenging since most patients experience only vomiting during the initial phase of their illness. When a norovirus infection is laboratory confirmed, vomiting has often resolved. Early sampling was easier during outbreaks, since more patients contracted illness sequentially. In that respect most of the samples collected from sporadic cases were in a later phase, and a longer time period had passed since the last vomiting event. But even samples collected in a later phase of disease were more likely positive during outbreaks. These differences in time periods since last vomiting event between outbreak and sporadic cases have biased the results making interpretation concerning relationship to outbreaks more difficult. The observed association between outbreaks and norovirus positive air still support the hypothesis that airborne dissemination to distant ward areas or possibly direct airborne transmission is of importance.

A few previous outbreak investigations have reached the conclusion that airborne transmission was a likely route [154-159]. The most discussed is an outbreak in a

hotel restaurant reported by Marks *et al* [157]. Analysis of the attack rate showed an inverse relationship with the distance from the index case who vomited, and other transmission routes seemed unlikely.

Limitations

Even if information about diarrhoea and vomiting was collected directly from the patient and /or ward staff, recollection, documentation and definition of these events pose challenges. The low number of patients and samples in this study hampered the possibility to conduct multivariable statistical analyses, controlling for more than one confounding variable. The absence of any positive air samples from sporadic cases without any connection to any ongoing of coming outbreaks also reduced the possibility to perform a proper statistical analysis of association to outbreaks, controlled for time since vomiting event. When interpreting the data it should be acknowledged that associations are not causations and viral RNA is not infectious virus particles.

Practical implications

The results suggest that airborne dissemination of noroviruses frequently occur during, and perhaps before, outbreaks. Control and optimization of ward air flow directions and ventilation rates may be relevant to prevent norovirus transmission. Considerations regarding additional personal protective equipment, such as respirators, may await further evidence of direct airborne transmission and estimations of (hypothetical) potential protective effect.

> "The best attempt at explaining the phenomena of cholera, which previously existed, was probably that which supposed that the disease was communicated by effluvia given off from the patient into the surrounding air, and inhaled by others into the lungs; but this view required its advocates to draw very largely on what is called predisposition, in order to account for the numbers who approach near to the patient without being affected, whilst others acquire the disease without any near approach."
>
> John Snow, "On the mode of communication of cholera", 1855

Transmission by environmental contamination

In paper IV aspects of environmental transmission was investigated. The aim was to study if admission to a room, previously occupied by a patient with norovirus infection infer a risk to the following patients admitted to that room.

All 21,371 patients admitted to any of the five Infectious Disease wards in Skåne between May 2013 and April 2018 were included in the cohort, comprising a total of 33,812 separate room stays. Norovirus infection was acquired by 54 patients during ward admission. Univariable and multivariable analysis was performed controlling for sharing room with any patient or sharing room with a patient with ongoing or recent (≤ 48 hours) symptoms, age and colonisations pressure (Table 10).

A high age was associated with norovirus acquisition, as was colonisation pressure at the ward. Sharing a room with any patient was associated with the outcome in the univariable analysis but not in a multivariable model. Sharing room with a patient with ongoing norovirus infection was, not surprisingly, associated with a high risk of norovirus acquisition.

Table 10. Risk factors associated with acquisition of NoV infection during room stay in univariable and multivariable analysis.

Study variable	Univariable analysis		Multivariable analysis[1]	
	OR (95% CI)	P	OR (95% CI)	P
Age, per year	1.03 (1.01-1.05)	0.001	1.02 (1.01-1.04)	0.006
Male sex	1.60 (0.92-2.80)	0.10		
Duration of room stay, per day	1.01 (0.98-1.04)	0.37		
Days with a roommate, per day	1.04 (1.00-1.07)	0.03		
Colonization pressure > 0 %[2]	15.0 (7.1-31.9)	<0.001	11.5 (5.3-24.8)	<0.001
Colonization pressure, per 10 % increase[3]	1.07 (1.01-1.12)	0.01		
Roommate with ongoing symptoms[4]	122 (40-369)	<0.001	35.7 (11.3-112.9)	<0.001
Prior room occupant with NoV infection[5]	4.76 (2.14-10.6)	<0.001	2.88 (1.28-6.46)	0.011

[1] Multivariable analysis using random effects model to account for ward dependency. Non-significant variables excluded from the analysis.
[2] Colonization pressure represents the cumulative proportion of patients with NoV infection at the ward during room stay.
[3]Calculated per 10% increase for exposed group only (colonization pressure > 0%) as continuous.
[4] Symptoms of NoV gastroenteritis ongoing or occurring up to < 48 hours.
[5] Admission to a room with a prior occupant with Nov infection, discharged the preceding 7 days

Room with a prior occupant with norovirus infection

Of the 1,031 admissions to a room with a prior occupant with norovirus infection in the preceding week, seven patients acquired norovirus infection during their room

stay. This exposure was significantly associated with norovirus infection acquisition in both uni- and multivariable analysis. A smaller sensitivity analysis, only including single occupancy rooms, did not alter the main result, albeit reached with a weaker probability (OR 2.6, p=0.086). The overall risk of acquiring norovirus infection during any Infectious Disease ward room stay was 0.2 % (54/33812) and the risk of acquiring norovirus infection during a stay in at room of a prior norovirus positive occupant was 0.7 % (7/1031), the attributable risk was 0.5 %, representing one infection in every 187 exposed room stays. Of all 54 acquired norovirus infections, environmental transmission within the room could be attributed to account for 5 cases, or 9 % of all ward acquired infections.

This risk of acquiring norovirus infection in a potentially contaminated room was similar to previous studies of various bacterial pathogens. In a recent meta-analysis of nine pooled studies, reported an overall odds ratio of 1.96 (1.36-2.68) of acquiring the studied pathogen from a prior room occupant [247]. In a previous study, pooled data from seven articles reached an overall odds ratio of 2.14 (1.65-2.77) [248].

The excess risk reported in Paper IV, emphasise the importance of cleaning at discharge. Hypochlorite is usually recommended for cleaning after environmental contamination of norovirus [210, 211]. In the studied wards Virkon® was used at terminal cleaning. An interpretation of the results is that Virkon® is an acceptable product for regular norovirus disinfection, but cleaning must be very thorough, at all times. If disinfection with hypochlorite would perform better in our setting is not known. Morter *et al.* found 45 % of the sampled surfaces positive for norovirus RNA after the first cleaning with hypochlorite, and 16 % after the second cleaning [224]. But positive tests may not be interpreted as infectious viruses, as hypochlorite is shown to efficiently inactivate noroviruses [142]

In recent years, there has been an increasing interest in "no-touch" disinfection methods in hope to reduce inadequate cleaning. The "no-touch" methods encompass disinfection with ultraviolet light (UV-C) and hydrogen peroxide systems [249]. Anderson *et al.* have reported that the addition of UV light to standard cleaning can decrease the risk of pathogen acquisition in a large cluster-randomized crossover study [250, 251]. If these concepts can reduce the risk of environmental transmission also of norovirus is not studied, but the methods have been evaluated in laboratory settings using surrogates for human norovirus [252, 253].

For limitations and implications, see "Transmission from asymptomatic patients".

> "The first case of decided Asiatic cholera in London, in the autumn of 1848, was that of a seaman named John Harnold, who had newly arrived by the Elbe steamer from Hamburgh, where the disease was prevailing.... He was seized with cholera on the 22nd of September, and died in a few hours... Now the next case of cholera, in London, occurred in the very room in which the above patient died. A man named Blenkinsopp came to lodge in the same room. He was attacked with cholera on the 30th September."

Comments of differences between wards

The number of ward acquired norovirus infections was much higher at Ward B and to some extent at Ward E (Table 11). The different epidemiology of the five included wards could not be explained by the variables in the statistical model. Compliance to standard and contact precautions, availability of single rooms, occupancy and staffing and perhaps ventilation might explain the differences observed, but requires further analysis.

Table 11. Characteristics of the included Infectious Disease wards (Unpublished data).

Variable	Ward				
	A	B	C	D	E
Beds (median)[1]	22	26	17	25	18
Rooms	22	24	16	17	14
Length of ward stay, mean	5.5	6.1	7.5	6.5	6.7
Room stays in single room %	99	54	70	31	29
Occupancy[1], mean %	95	100	98	96	100
Compliance standard precautions, mean %	98	92	88	89	80
Ward admissions of patients with non-ward acquired NoV infection (%[2])	82 (1.4)	99 (1.1)	32 (0.8)	72 (1.1)	41 (0.8)
Colonization pressure, mean %	5.3	7.8	2.5	5.2	4.8
Ward acquired NoV infection (‰[3])	6 (0.9)	31 (3.4)	2 (0.4)	4 (0.5)	11 (1.9)

[1]Sources to beds and occupance from data in management and control database accessed by Qlickview..

[2] % of total admissions

[3] ‰ of total room stays

Transmission from asymptomatic patients

There is substantial evidence of transmission of norovirus from asymptomatic food-handlers during food preparation [198]. In healthcare, examples of transmission from asymptomatic patients are rare. Patients with chronic shedding, but not entirely asymptomatic, have been shown to transmit norovirus [133]. Asymptomatic norovirus carriers are not necessarily admitted to single occupancy rooms and in patients with symptomatic infection, isolation is usually terminated 48 hours after the symptoms have resolved, even though viral shedding in high concentration is likely to continue for at least two or three more weeks [99].

In Paper IV, as a secondary aim, we investigated if sharing room with a patient with resolved symptoms of norovirus infection (since ≥ 48 hours) entailed a risk for norovirus acquisition. During 53 room stays patients shared room with another patient with symptom resolution since ≥ 48 hours and none contracted norovirus infection. The result indicates that discontinuation of isolation precautions, according too current routine, is adequate.

A closely related possible interpretation is also that patients with asymptomatic norovirus infection transfer disease only rarely in hospital settings. The carrier status of the patients with resolved infection in the current study was not known, but as discussed previously, shedding during the studied intervals was likely. Sukhire *et al.* constructed plausible transmission pathways based on shedding kinetics in extensively investigated outbreaks where both symptomatic and asymptomatic patients and HCW were tested [254]. The conclusion was that symptomatic cases were more often involved in transmission events than asymptomatic cases, but asymptomatic patients contributed to transmission and were estimated to carry an estimated average reproduction number (R_{eff}) of 0.85 (CI 95% 0.55-1.05). Possible transmission from environmental sources was not included in the transmission model. The number of exposed in our study was small and we only had 80 % power to detect an odds ratio of approximately 20, but still enough to question the generalizability of the reproductive number reached by Sukire *et al.*

Limitation

Paper IV has several limitations. Patients with undetected symptomatic and asymptomatic norovirus infection are likely to have occurred at the wards since norovirus tests were ordered at the discretion of the physicians. The study was conducted at Infectious Disease ward partly based on a non-validated assumption that these wards might have a higher level attention to gastroenteritis symptoms and a lower threshold for norovirus sampling.

The follow up period after ward discharge was limited to any norovirus test sampled at the following ward if the patient was transfer within hospitals, or in primary care.

As has been discussed in relation to Paper II, medical records are not a perfect data source of details about gastrointestinal symptoms. Documentation was still good enough for the definitions used, although data collection entailed some uncertainty of exact time of onset and resolution. Data on time of admission and discharge is entered to the database by ward staff and also incorporate some incorrect data. The admission data was analysed to find and exclude such incorrect information, by cross-checking for simultaneous room stays and very long room stays. Sixty inconsistent room stays were excluded. Nor might the time of admission and discharge and duration of room stay always correspond to the time spent in the room. If a patient is transferred for a short time period, for example for a surgical procedure, the patient is not transferred in the room database, and sometimes the real admission and discharge time differ a few hours from the documented time. We choose to use a minimum exposure time of three hours, which is still relatively short, to include as many exposures as possible.

Practical implications

The implications of the result concerning risk of prior occupant is that cleaning quality must be excellent to avoid environmental transmission within the room. Considerations of a switch to hypochlorite or perhaps investigate the possibility of implementing a no-touch disinfection method is justified, but Virkon® seems adequate if cleaning quality can be ensured. The routine of discontinuation of isolation ≥ 48 hours after resolution of symptoms seems safe.

Future perspectives on the mode of communication of norovirus

In this thesis, aspects of surveillance and factors associated to transmission and outbreak development have been explored.

We have seen a lower level of norovirus activity the past six years, but we cannot make predictions for the future. And, even during low incidence years, norovirus continue to cause outbreaks, with consequences for frail hospitalised patients, staff and healthcare management. An explanatory framework of how transmission occurs is crucial to improve preventive actions.

Suggested framework for the success of GII.4 variants

We have seen how the successful GII.4 genotype, with an improved capability to mutate or recombine to new variants, repeatedly can evade herd immunity and cause global pandemics. These variants seem to have a varying degree of increased virulence, causing a more severe disease with more frequent vomiting. These vomits seem to be the key in the successful adaptation to efficient transmission within the large epidemiologic niche of modern semi-enclosed settings, including hospitals and nursing homes. Current research suggests that the low outside absolute humidity during the cold winter season correlates with low indoor relative humidity and this low relative humidity might enhance the formation of virus containing aerosol during vomiting events. The aerosol droplet nuclei can disseminate and settle at distant surfaces and contribute to environmental transmission or may be inhaled and be deposited in the upper respiratory tract and swallowed.

Hypotheses concerning transmission in hospitals

The number and proportion of affected healthcare is high during norovirus outbreaks, higher than for most other infectious diseases. This could have several different, non-conflicting, explanations. If all implications of possible occurrence of viable airborne norovirus are to be excluded, this observation would have to be explained by inadequate hand hygiene or improper use of gloves and gowns during and after care within the room of the norovirus patients. The insufficiently cleaned hands then transmit the virus to other surfaces within the ward, explaining the widespread contamination of surfaces observed in several studies, and the virus

eventually reaches the mouth. An alternate explanation, assuming compliance to hand hygiene and personal protective equipment is near perfect, would be to acknowledge airborne dissemination to distant surfaces as an important transmission route. Contact environmental transmission would then occur from fomites, never suspected to be contaminated. A third possible explanation would be that direct airborne transmission frequently occurs. Further studies to better understand and verify any of these hypothetical explanations are essential for improved future prevention and reduced occupational transmission risks.

Airborne transmission of noroviruses?

We still lack some pieces of evidence to prove direct airborne transmission. We have examples of outbreaks in which airborne transmission was considered the most likely pathway, we have detected norovirus RNA in air in sufficient concentration to cause disease and we have experimental evidence that human norovirus surrogates (which in most cases seem more fragile than human noroviruses) can preserve their infectivity during aerosolization. We lack evidence that human noroviruses are still infectious in sufficient concentrations in aerosol and we lack direct aerosol challenge studies. We also lack any real world estimates regarding if airborne transmission is just a possibility but very rarely occurring or if it is a common mode of transmission. Additional studies to address the remaining issues concerning possible airborne transmission of noroviruses are warranted.

Contagious diseases have been believed to be transmitted by foul air (miasma or effluvia), supposedly polluted by ill people under poor hygienic conditions or sewage, since Hippocrates further during the middle ages [255]. The investigations by John Snow, and the following work by William Farr, finally convinced the scientific society that the miasma theory was wrong. In recent years, as pointed out by Roy *et al.,* the tide has turned [256]. The support and interest for airborne transmission and dissemination as a possible adjunctive transmission route for various infectious diseases, is growing [257-259]. Cholera is not (yet?) found in air, but for norovirus the miasma circle is closing. Even sewage might hypothetically be implicated in airborne transmission of norovirus, as norovirus recently was found in wastewater treatment plant air [260].

Prevention of possible airborne transmission

Indoor air ventilation rate and directional airflow are likely to influence possible airborne transmission and dissemination. Improvement of ventilation might help prevent norovirus transmission in semi-enclosed settings, but further studies are necessary. Admission of patients with suspected norovirus gastroenteritis to rooms ventilated at negative pressure relative to the ward corridor and use of respirators when entering might also be considered. Such a recommendation currently lack sufficient evidence. Estimations of any possible protective effect of such methods,

enabling a cost-benefit analysis would be desirable. Prevention of aerosol formation might be a possibility if vomiting could be reduced with antiemetic drugs. Further studies are needed to answer these questions.

Prevention of environmental transmission

All attempts to estimate and model norovirus transmission conclude that environmental transmission is the most important transmission route [261-263]. Hospital design, specifically, to a larger extent construct and use single rooms is likely to prevent environmental transmission and outbreaks. England, where large open patient bays are frequent have had a high outbreak incidence in hospitals. A shift to more single rooms have been reported to reduce to outbreaks incidence [264]. The differences noted between the risks of norovirus acquisition in different wards in Paper IV might also partly be explained by the different availability of single rooms.

Environmental contamination is shown to be a common finding and is often perceived as an important factor in outbreak reports. In Paper IV, implied environmental contamination was associated with transmission in a setting without large outbreaks. The support for recommending a more active approach for cleaning and disinfection is growing. An increased frequency of cleaning and disinfection at high-touch surfaces within and outside, the rooms of sporadic and outbreak norovirus cases, would probably be beneficial. An improved cleaning and disinfection efficacy is also likely to better prevent transmission and introduction of "no-touch" methods might reduce environmental contamination from prior room occupants and others.

Prevention of norovirus outbreaks and transmission

Successful prevention is likely to incorporate a multidimensional approach, including hospital design, organisational preparedness and education, vigilant staff with high adherence to recommended routines, a secure surveillance system, preferably all admission only to single rooms, appropriate ventilation system and a meticulous cleaning organisation, using efficient methods for cleaning and disinfection.

> "I feel confident, however, that by attending to the above-mentioned precautions, which I consider to be based on a correct knowledge of the cause of cholera, this disease may be rendered extremely rare, if indeed it may not be altogether banished from civilized countries."
>
> John Snow, "On the mode of communication of cholera", 1855

Conclusions

- Norovirus outbreaks in hospitals are common during the winter seasons. The outbreak incidence hospitals wards in Skåne 2010-2012 varied between 0.5 and 0.2 outbreaks per ward and season, during high and low incidence seasons, respectively.
- Laboratory surveillance performed better than clinical surveillance in detecting outbreaks. Combined clinical and laboratory surveillance, including admission data is the best alternative.
- Patients with norovirus infections are more likely to transmit disease and start an outbreak if they vomit, are sharing room with other patients, are of old age (> 80 years or age), have comorbidities or have onset of disease at the ward.
- Infection with norovirus GII.4 genotype is associated with an increased risk of outbreak development and, with vomiting, implying a possible explanation for the epidemiological connection between GII.4 genotype and outbreaks in hospitals.
- The detection of norovirus RNA in the air is associated with a shorter period of time since the last vomiting episode. This association suggests vomiting as the major source of airborne norovirus.
- The presence and concentration of norovirus RNA in submicrometre particles indicates that airborne transmission is a possibility.
- Airborne norovirus RNA is a common finding during outbreaks. The association between airborne norovirus RNA and outbreaks supports the hypothesis that the airborne transmission and dissemination route may be of importance.
- A prior room occupant with norovirus infection is associated with a risk of norovirus acquisition for the following patients in that room. The risk is low in absolute terms but the association emphasise the importance of cleaning and disinfection to prevent environmental transmission of norovirus.
- Transmission from roommates with resolved symptoms since more than 48 hours seems rare.

Acknowledgments

This thesis has been accomplished by the contribution, help and support of many others. I would like to express my deepest gratitude to everyone, in anyway, involved. In particular, I want to thank:

Blenda Böttiger, my main supervisor, for accepting me as a PhD student when you arrived in Lund. You have been the backbone of my research project. Always knowledgeable, perceptive and caring.

Malin Inghammar, my main co-supervisor, for your absolutely invaluable support, energy and impressive skills in guiding this project through all epidemiological, statistical and administrative burns and cuts.

Hugo Johansson, my start up co-supervisor, for inspiring and supporting me to start this project and follow it all the way through, in your very humble way.

The Infection Control team, doctors and nurses, for cheering and supporting me in all possible ways, including dedicated outbreak data collection. An extra special thanks to Ulf Karlsson for versatile discussions about the state of things.

Jakob Löndahl and Malin Alsved, for a close and fruitful research collaboration. For too long I have passed the Aerosol Laboratory wondering what they were doing in there. I am truly grateful we finally got in touch.

Anna Söderlund-Strand, for methodological development, analytic support and valuable comments. I thank all other contributors and co-authors, Mats Bohgard, Anders Widell, Torsten Holmdahl, Peter Lanbeck, Anders Gudmundsson and Patrik Medstrand for great interdisciplinary discussions and insightful comments. Also thanks to Sara Hassan for skilled laboratory assistance.

All colleagues and staff at the Department of Infectious Diseases for creating an open, warm and friendly atmosphere.

Friends, for understanding or not understanding.

My sister, Clara, for all help and commitment, including with the cover design.

My parents, Paula and Lars, for your love, inspiration and unconditional support.

My fantastic family; beloved wife, Charlotta, and sons, Gustav and Axel, for enduring research and, for all that deeply matters.

There are more people to thank; administrative facilitators, Swedish Infection Control colleagues, former Infectious Disease colleagues, former research partners, and more, you are not forgotten.

I would also like to thank all patients involved. You have not been asked for permission, but I have used some of your medical data, and collected some air….

This thesis has been continuously supported by grants from Södra Regionvårdnämnden. Additional support has been granted from Helene Marckwardts foundation, Swedish Research Council FORMAS, Swedish Research Council Vetenskapsrådet and AFA Insurance.

References

1. Zahorsky J. Hyperemesis hiemis or the winter vomiting disease. Arch Pediat **1929**; 46: 391-5.
2. Henningsen E. En ejendommelig epidemi i Roskilde december 1935. Ugeskrift for Læger **1936**; 98(3): 45-8.
3. Adler JL, Zickl R. Winter vomiting disease. J Infect Dis **1969**; 119(6): 668-73.
4. Dolin R, Blacklow NR, DuPont H, et al. Transmission of acute infectious nonbacterial gastroenteritis to volunteers by oral administration of stool filtrates. J Infect Dis **1971**; 123(3): 307-12.
5. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol **1972**; 10(5): 1075-81.
6. Kaplan JE, Gary GW, Baron RC, et al. Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann Intern Med **1982**; 96(6 Pt 1): 756-61.
7. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. Am J Public Health **1982**; 72(12): 1329-32.
8. Xi JN, Graham DY, Wang KN, Estes MK. Norwalk virus genome cloning and characterization. Science **1990**; 250(4987): 1580-3.
9. Jiang X, Wang J, Graham DY, Estes MK. Detection of Norwalk virus in stool by polymerase chain reaction. J Clin Microbiol **1992**; 30(10): 2529-34.
10. Glass RI, Noel J, Ando T, et al. The epidemiology of enteric caliciviruses from humans: a reassessment using new diagnostics. J Infect Dis **2000**; 181 Suppl 2: S254-61.
11. Siebenga JJ, Lemey P, Kosakovsky Pond SL, Rambaut A, Vennema H, Koopmans M. Phylodynamic reconstruction reveals norovirus GII.4 epidemic expansions and their molecular determinants. PLoS Pathog **2010**; 6(5): e1000884.
12. Ettayebi K, Crawford SE, Murakami K, et al. Replication of human noroviruses in stem cell-derived human enteroids. Science **2016**; 353(6306): 1387-93.
13. Estes MK, Ettayebi K, Tenge VR, et al. Human Norovirus Cultivation in Nontransformed Stem Cell-Derived Human Intestinal Enteroid Cultures: Success and Challenges. Viruses **2019**; 11(7).

14. Lopman B, Vennema H, Kohli E, et al. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet **2004**; 363(9410): 682-8.
15. Ahmed SM, Hall AJ, Robinson AE, et al. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis **2014**; 14(8): 725-30.
16. Bartsch SM, Lopman BA, Ozawa S, Hall AJ, Lee BY. Global Economic Burden of Norovirus Gastroenteritis. PLoS One **2016**; 11(4): e0151219.
17. Thorne LG, Goodfellow IG. Norovirus gene expression and replication. J Gen Virol **2014**; 95(Pt 2): 278-91.
18. Smith HQ, Smith TJ. The Dynamic Capsid Structures of the Noroviruses. Viruses **2019**; 11(3).
19. Donaldson EF, Lindesmith LC, Lobue AD, Baric RS. Viral shape-shifting: norovirus evasion of the human immune system. Nat Rev Microbiol **2010**; 8(3): 231-41.
20. Conley MJ, McElwee M, Azmi L, et al. Calicivirus VP2 forms a portal-like assembly following receptor engagement. Nature **2019**; 565(7739): 377-81.
21. Oka T, Wang Q, Katayama K, Saif LJ. Comprehensive review of human sapoviruses. Clin Microbiol Rev **2015**; 28(1): 32-53.
22. Desselberger U. Caliciviridae Other Than Noroviruses. Viruses **2019**; 11(3).
23. Cortes-Penfield NW, Ramani S, Estes MK, Atmar RL. Prospects and Challenges in the Development of a Norovirus Vaccine. Clin Ther **2017**; 39(8): 1537-49.
24. Chhabra P, de Graaf M, Parra GI, et al. Updated classification of norovirus genogroups and genotypes. J Gen Virol **2019**.
25. Kniel KE. The makings of a good human norovirus surrogate. Curr Opin Virol **2014**; 4: 85-90.
26. Drake JW, Holland JJ. Mutation rates among RNA viruses. Proc Natl Acad Sci U S A **1999**; 96(24): 13910-3.
27. Bull RA, Eden JS, Rawlinson WD, White PA. Rapid evolution of pandemic noroviruses of the GII.4 lineage. PLoS Pathog **2010**; 6(3): e1000831.
28. Parra GI, Squires RB, Karangwa CK, et al. Static and Evolving Norovirus Genotypes: Implications for Epidemiology and Immunity. PLoS Pathog **2017**; 13(1): e1006136.
29. de Graaf M, van Beek J, Koopmans MP. Human norovirus transmission and evolution in a changing world. Nat Rev Microbiol **2016**; 14(7): 421-33.
30. Atmar RL, Ramani S, Estes MK. Human noroviruses: recent advances in a 50-year history. Curr Opin Infect Dis **2018**; 31(5): 422-32.
31. de Graaf M, van Beek J, Vennema H, et al. Emergence of a novel GII.17 norovirus - End of the GII.4 era? Euro Surveill **2015**; 20(26).
32. Zou WY, Blutt SE, Crawford SE, et al. Human Intestinal Enteroids: New Models to Study Gastrointestinal Virus Infections. Methods Mol Biol **2017**.

33. Karandikar UC, Crawford SE, Ajami NJ, et al. Detection of human norovirus in intestinal biopsies from immunocompromised transplant patients. J Gen Virol **2016**; 97(9): 2291-300.
34. Troeger H, Loddenkemper C, Schneider T, et al. Structural and functional changes of the duodenum in human norovirus infection. Gut **2009**; 58(8): 1070-7.
35. Karst SM, Tibbetts SA. Recent advances in understanding norovirus pathogenesis. J Med Virol **2016**; 88(11): 1837-43.
36. Karst SM, Wobus CE. A working model of how noroviruses infect the intestine. PLoS Pathog **2015**; 11(2): e1004626.
37. Hagbom M, Istrate C, Engblom D, et al. Rotavirus stimulates release of serotonin (5-HT) from human enterochromaffin cells and activates brain structures involved in nausea and vomiting. PLoS Pathog **2011**; 7(7): e1002115.
38. Hagbom M, Novak D, Ekstrom M, et al. Ondansetron treatment reduces rotavirus symptoms-A randomized double-blinded placebo-controlled trial. PLoS One **2017**; 12(10): e0186824.
39. Nordgren J, Svensson L. Genetic Susceptibility to Human Norovirus Infection: An Update. Viruses **2019**; 11(3).
40. Lindesmith L, Moe C, Marionneau S, et al. Human susceptibility and resistance to Norwalk virus infection. Nat Med **2003**; 9(5): 548-53.
41. Lopman BA, Trivedi T, Vicuna Y, et al. Norovirus Infection and Disease in an Ecuadorian Birth Cohort: Association of Certain Norovirus Genotypes With Host FUT2 Secretor Status. J Infect Dis **2015**; 211(11): 1813-21.
42. Nordgren J, Sharma S, Kambhampati A, Lopman B, Svensson L. Innate Resistance and Susceptibility to Norovirus Infection. PLoS Pathog **2016**; 12(4): e1005385.
43. Hutson AM, Atmar RL, Graham DY, Estes MK. Norwalk virus infection and disease is associated with ABO histo-blood group type. J Infect Dis **2002**; 185(9): 1335-7.
44. Parrino TA, Schreiber DS, Trier JS, Kapikian AZ, Blacklow NR. Clinical immunity in acute gastroenteritis caused by Norwalk agent. N Engl J Med **1977**; 297(2): 86-9.
45. Johnson PC, Mathewson JJ, DuPont HL, Greenberg HB. Multiple-challenge study of host susceptibility to Norwalk gastroenteritis in US adults. J Infect Dis **1990**; 161(1): 18-21.
46. Simmons K, Gambhir M, Leon J, Lopman B. Duration of immunity to norovirus gastroenteritis. Emerg Infect Dis **2013**; 19(8): 1260-7.
47. Ramani S, Neill FH, Opekun AR, et al. Mucosal and Cellular Immune Responses to Norwalk Virus. J Infect Dis **2015**; 212(3): 397-405.
48. Shanker S, Czako R, Sapparapu G, et al. Structural basis for norovirus neutralization by an HBGA blocking human IgA antibody. Proc Natl Acad Sci U S A **2016**; 113(40): E5830-E7.

49. Reeck A, Kavanagh O, Estes MK, et al. Serological correlate of protection against norovirus-induced gastroenteritis. J Infect Dis **2010**; 202(8): 1212-8.
50. Woodward J, Gkrania-Klotsas E, Kumararatne D. Chronic norovirus infection and common variable immunodeficiency. Clin Exp Immunol **2017**; 188(3): 363-70.
51. Chong PP, Atmar RL. Norovirus in health care and implications for the immunocompromised host. Curr Opin Infect Dis **2019**; 32(4): 348-55.
52. Lindesmith LC, Mallory ML, Jones TA, et al. Impact of Pre-exposure History and Host Genetics on Antibody Avidity Following Norovirus Vaccination. J Infect Dis **2017**; 215(6): 984-91.
53. Saito M, Goel-Apaza S, Espetia S, et al. Multiple norovirus infections in a birth cohort in a Peruvian Periurban community. Clin Infect Dis **2014**; 58(4): 483-91.
54. Sakon N, Yamazaki K, Nakata K, et al. Impact of genotype-specific herd immunity on the circulatory dynamism of norovirus: a 10-year longitudinal study of viral acute gastroenteritis. J Infect Dis **2015**; 211(6): 879-88.
55. Kim L, Liebowitz D, Lin K, et al. Safety and immunogenicity of an oral tablet norovirus vaccine, a phase I randomized, placebo-controlled trial. JCI Insight **2018**; 3(13).
56. Atmar RL, Baehner F, Cramer JP, et al. Antibody persistence to two Virus-Like Particle norovirus vaccine candidate formulations in healthy adults: one-year follow-up with memory probe vaccination. J Infect Dis **2019**.
57. Leroux-Roels G, Cramer JP, Mendelman PM, et al. Safety and Immunogenicity of Different Formulations of Norovirus Vaccine Candidate in Healthy Adults: A Randomized, Controlled, Double-Blind Clinical Trial. J Infect Dis **2018**; 217(4): 597-607.
58. Bernstein DI, Atmar RL, Lyon GM, et al. Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults. J Infect Dis **2015**; 211(6): 870-8.
59. Lee RM, Lessler J, Lee RA, et al. Incubation periods of viral gastroenteritis: a systematic review. BMC Infect Dis **2013**; 13: 446.
60. Atmar RL, Opekun AR, Gilger MA, et al. Determination of the 50% human infectious dose for Norwalk virus. J Infect Dis **2014**; 209(7): 1016-22.
61. Teunis PF, Moe CL, Liu P, et al. Norwalk virus: how infectious is it? J Med Virol **2008**; 80(8): 1468-76.
62. Kirby AE, Teunis PF, Moe CL. Two human challenge studies confirm high infectivity of Norwalk virus. J Infect Dis **2015**; 211(1): 166-7.
63. Santiana M, Ghosh S, Ho BA, et al. Vesicle-Cloaked Virus Clusters Are Optimal Units for Inter-organismal Viral Transmission. Cell Host Microbe **2018**; 24(2): 208-20 e8.
64. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Engl J Med **2009**; 361(18): 1776-85.
65. Rockx B, De Wit M, Vennema H, et al. Natural history of human calicivirus infection: a prospective cohort study. Clin Infect Dis **2002**; 35(3): 246-53.

66. Arias C, Sala MR, Dominguez A, et al. Epidemiological and clinical features of norovirus gastroenteritis in outbreaks: a population-based study. Clin Microbiol Infect **2010**; 16(1): 39-44.
67. Lopman BA, Reacher MH, Vipond IB, Sarangi J, Brown DW. Clinical manifestation of norovirus gastroenteritis in health care settings. Clin Infect Dis **2004**; 39(3): 318-24.
68. Sala MR, Broner S, Moreno A, et al. Cases of acute gastroenteritis due to calicivirus in outbreaks: clinical differences by age and aetiological agent. Clin Microbiol Infect **2014**; 20(8): 793-8.
69. Gotz H, Ekdahl K, Lindback J, de Jong B, Hedlund KO, Giesecke J. Clinical spectrum and transmission characteristics of infection with Norwalk-like virus: findings from a large community outbreak in Sweden. Clin Infect Dis **2001**; 33(5): 622-8.
70. Partridge DG, Evans CM, Raza M, Kudesia G, Parsons HK. Lessons from a large norovirus outbreak: impact of viral load, patient age and ward design on duration of symptoms and shedding and likelihood of transmission. J Hosp Infect **2012**; 81(1): 25-30.
71. Friesema IH, Vennema H, Heijne JC, et al. Differences in clinical presentation between norovirus genotypes in nursing homes. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **2009**; 46(4): 341-4.
72. Rosenthal NA, Lee LE, Vermeulen BA, et al. Epidemiological and genetic characteristics of norovirus outbreaks in long-term care facilities, 2003-2006. Epidemiol Infect **2011**; 139(2): 286-94.
73. Desai R, Hembree CD, Handel A, et al. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. Clin Infect Dis **2012**; 55(2): 189-93.
74. Swartling L, Ljungman P, Remberger M, et al. Norovirus causing severe gastrointestinal disease following allogeneic hematopoietic stem cell transplantation: A retrospective analysis. Transpl Infect Dis **2018**; 20(2): e12847.
75. Brakemeier S, Taxeidi SI, Durr M, et al. Clinical outcome of norovirus infection in renal transplant patients. Clin Transplant **2016**; 30(10): 1283-93.
76. Rolfes MC, Sriaroon P, Davila Saldana BJ, et al. Chronic norovirus infection in primary immune deficiency disorders: an international case series. Diagn Microbiol Infect Dis **2019**; 93(1): 69-73.
77. Stuart RL, Tan K, Mahar JE, et al. An outbreak of necrotizing enterocolitis associated with norovirus genotype GII.3. Pediatr Infect Dis J **2010**; 29(7): 644-7.
78. Turcios-Ruiz RM, Axelrod P, St John K, et al. Outbreak of necrotizing enterocolitis caused by norovirus in a neonatal intensive care unit. J Pediatr **2008**; 153(3): 339-44.

79. Hu MH, Lin KL, Wu CT, Chen SY, Huang GS. Clinical Characteristics and Risk Factors for Seizures Associated With Norovirus Gastroenteritis in Childhood. J Child Neurol **2017**; 32(9): 810-4.
80. Castellazzi L, Principi N, Agostoni C, Esposito S. Benign convulsions in children with mild gastroenteritis. Eur J Paediatr Neurol **2016**; 20(5): 690-5.
81. Mattner F, Sohr D, Heim A, Gastmeier P, Vennema H, Koopmans M. Risk groups for clinical complications of norovirus infections: an outbreak investigation. Clin Microbiol Infect **2006**; 12(1): 69-74.
82. Veno S, Eckardt J. Boerhaave's syndrome and tension pneumothorax secondary to Norovirus induced forceful emesis. J Thorac Dis **2013**; 5(2): E38-40.
83. Pawa N, Vanezis AP, Tutton MG. Spontaneous bowel perforation due to norovirus: a case report. Cases J **2009**; 2: 9101.
84. Ueda N. Gastroduodenal Perforation and Ulcer Associated With Rotavirus and Norovirus Infections in Japanese Children: A Case Report and Comprehensive Literature Review. Open Forum Infect Dis **2016**; 3(1): ofw026.
85. Trivedi TK, Desai R, Hall AJ, Patel M, Parashar UD, Lopman BA. Clinical characteristics of norovirus-associated deaths: a systematic literature review. Am J Infect Control **2013**; 41(7): 654-7.
86. Banyai K, Estes MK, Martella V, Parashar UD. Viral gastroenteritis. Lancet **2018**; 392(10142): 175-86.
87. Pires SM, Fischer-Walker CL, Lanata CF, et al. Aetiology-Specific Estimates of the Global and Regional Incidence and Mortality of Diarrhoeal Diseases Commonly Transmitted through Food. PLoS One **2015**; 10(12): e0142927.
88. Hall AJ, Lopman BA, Payne DC, et al. Norovirus disease in the United States. Emerg Infect Dis **2013**; 19(8): 1198-205.
89. Harris JP, Edmunds WJ, Pebody R, Brown DW, Lopman BA. Deaths from norovirus among the elderly, England and Wales. Emerg Infect Dis **2008**; 14(10): 1546-52.
90. Hall AJ, Curns AT, McDonald LC, Parashar UD, Lopman BA. The roles of Clostridium difficile and norovirus among gastroenteritis-associated deaths in the United States, 1999-2007. Clin Infect Dis **2012**; 55(2): 216-23.
91. Trivedi TK, DeSalvo T, Lee L, et al. Hospitalizations and mortality associated with norovirus outbreaks in nursing homes, 2009-2010. JAMA **2012**; 308(16): 1668-75.
92. Gustavsson L, Andersson LM, Lindh M, Westin J. Excess mortality following community-onset norovirus enteritis in the elderly. J Hosp Infect **2011**; 79(1): 27-31.
93. Gallimore CI, Cubitt D, du Plessis N, Gray JJ. Asymptomatic and symptomatic excretion of noroviruses during a hospital outbreak of gastroenteritis. J Clin Microbiol **2004**; 42(5): 2271-4.

94. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. N Engl J Med **2011**; 365(23): 2178-87.
95. Currier RL, Payne DC, Staat MA, et al. Innate Susceptibility to Norovirus Infections Influenced by FUT2 Genotype in a United States Pediatric Population. Clin Infect Dis **2015**; 60(11): 1631-8.
96. Matthews JE, Dickey BW, Miller RD, et al. The epidemiology of published norovirus outbreaks: a review of risk factors associated with attack rate and genogroup. Epidemiol Infect **2012**; 140(7): 1161-72.
97. Atmar RL, Bernstein DI, Lyon GM, et al. Serological Correlates of Protection against a GII.4 Norovirus. Clin Vaccine Immunol **2015**; 22(8): 923-9.
98. Atmar RL, Opekun AR, Gilger MA, et al. Norwalk virus shedding after experimental human infection. Emerg Infect Dis **2008**; 14(10): 1553-7.
99. Teunis PF, Sukhrie FH, Vennema H, Bogerman J, Beersma MF, Koopmans MP. Shedding of norovirus in symptomatic and asymptomatic infections. Epidemiol Infect **2015**; 143(8): 1710-7.
100. Kirby AE, Streby A, Moe CL. Vomiting as a Symptom and Transmission Risk in Norovirus Illness: Evidence from Human Challenge Studies. PLoS One **2016**; 11(4): e0143759.
101. Lee N, Chan MC, Wong B, et al. Fecal viral concentration and diarrhea in norovirus gastroenteritis. Emerg Infect Dis **2007**; 13(9): 1399-401.
102. Kirby AE, Shi J, Montes J, Lichtenstein M, Moe CL. Disease course and viral shedding in experimental Norwalk virus and Snow Mountain virus infection. J Med Virol **2014**; 86(12): 2055-64.
103. Aoki Y, Suto A, Mizuta K, Ahiko T, Osaka K, Matsuzaki Y. Duration of norovirus excretion and the longitudinal course of viral load in norovirus-infected elderly patients. J Hosp Infect **2010**; 75(1): 42-6.
104. Lai CC, Wang YH, Wu CY, Hung CH, Jiang DD, Wu FT. A norovirus outbreak in a nursing home: norovirus shedding time associated with age. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **2013**; 56(2): 96-101.
105. Murata T, Katsushima N, Mizuta K, Muraki Y, Hongo S, Matsuzaki Y. Prolonged norovirus shedding in infants <or=6 months of age with gastroenteritis. Pediatr Infect Dis J **2007**; 26(1): 46-9.
106. Phillips G, Tam CC, Rodrigues LC, Lopman B. Prevalence and characteristics of asymptomatic norovirus infection in the community in England. Epidemiol Infect **2010**; 138(10): 1454-8.
107. Qi R, Huang YT, Liu JW, et al. Global Prevalence of Asymptomatic Norovirus Infection: A Meta-analysis. EClinicalMedicine **2018**; 2-3: 50-8.
108. Kaarme J, Hickman RA, Neveus T, Blomberg J, Ohrmalm C. Reassuringly low carriage of enteropathogens among healthy Swedish children in day care centres. Public Health **2016**; 140: 221-7.
109. Vinje J. Advances in laboratory methods for detection and typing of norovirus. J Clin Microbiol **2015**; 53(2): 373-81.

110. Zhuo R, Cho J, Qiu Y, et al. High genetic variability of norovirus leads to diagnostic test challenges. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **2017**; 96: 94-8.
111. Kageyama T, Kojima S, Shinohara M, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol **2003**; 41(4): 1548-57.
112. Gustavsson L, Westin J, Andersson LM, Lindh M. Rectal swabs can be used for diagnosis of viral gastroenteritis with a multiple real-time PCR assay. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **2011**; 51(4): 279-82.
113. Kirby A, Dove W, Ashton L, Hopkins M, Cunliffe NA. Detection of norovirus in mouthwash samples from patients with acute gastroenteritis. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **2010**; 48(4): 285-7.
114. Franck KT, Fonager J, Ersboll AK, Bottiger B. Norovirus epidemiology in community and health care settings and association with patient age, Denmark. Emerg Infect Dis **2014**; 20(7): 1123-31.
115. Haubrich K, Gantt S, Blydt-Hansen T. Successful treatment of chronic norovirus gastroenteritis with nitazoxanide in a pediatric kidney transplant recipient. Pediatr Transplant **2018**; 22(4): e13186.
116. Kempf B, Edgar JD, Mc Caughey C, Devlin LA. Nitazoxanide Is an Ineffective Treatment of Chronic Norovirus in Patients With X-Linked Agammaglobulinemia and May Yield False-Negative Polymerase Chain Reaction Findings in Stool Specimens. J Infect Dis **2017**; 215(3): 486-7.
117. Rossignol JF, El-Gohary YM. Nitazoxanide in the treatment of viral gastroenteritis: a randomized double-blind placebo-controlled clinical trial. Aliment Pharmacol Ther **2006**; 24(10): 1423-30.
118. Brown LK, Clark I, Brown JR, Breuer J, Lowe DM. Norovirus infection in primary immune deficiency. Rev Med Virol **2017**; 27(3): e1926.
119. Lopman BA, Hall AJ, Curns AT, Parashar UD. Increasing rates of gastroenteritis hospital discharges in US adults and the contribution of norovirus, 1996-2007. Clin Infect Dis **2011**; 52(4): 466-74.
120. Phillips G, Tam CC, Conti S, et al. Community incidence of norovirus-associated infectious intestinal disease in England: improved estimates using viral load for norovirus diagnosis. Am J Epidemiol **2010**; 171(9): 1014-22.
121. Tam CC, Rodrigues LC, Viviani L, et al. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut **2012**; 61(1): 69-77.
122. Verstraeten T, Cattaert T, Harris J, Lopman B, Tam CC, Ferreira G. Estimating the Burden of Medically Attended Norovirus Gastroenteritis: Modeling Linked Primary Care and Hospitalization Datasets. J Infect Dis **2017**; 216(8): 957-65.

123. Verhoef L, Koopmans M, W VANP, et al. The estimated disease burden of norovirus in The Netherlands. Epidemiol Infect **2013**; 141(3): 496-506.
124. O'Brien SJ, Donaldson AL, Iturriza-Gomara M, Tam CC. Age-Specific Incidence Rates for Norovirus in the Community and Presenting to Primary Healthcare Facilities in the United Kingdom. J Infect Dis **2016**; 213 Suppl 1: S15-8.
125. Hedlund KO, Rubilar-Abreu E, Svensson L. Epidemiology of calicivirus infections in Sweden, 1994-1998. J Infect Dis **2000**; 181 Suppl 2: S275-80.
126. Johansen K, Mannerqvist K, Allard A, et al. Norovirus strains belonging to the GII.4 genotype dominate as a cause of nosocomial outbreaks of viral gastroenteritis in Sweden 1997--2005. Arrival of new variants is associated with large nation-wide epidemics. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology **2008**; 42(2): 129-34.
127. Edelstein M, Wallensten A, Zetterqvist I, Hulth A. Detecting the norovirus season in Sweden using search engine data--meeting the needs of hospital infection control teams. PLoS One **2014**; 9(6): e100309.
128. van Beek J, de Graaf M, Al-Hello H, et al. Molecular surveillance of norovirus, 2005-16: an epidemiological analysis of data collected from the NoroNet network. Lancet Infect Dis **2018**; 18(5): 545-53.
129. Mounts AW, Ando T, Koopmans M, Bresee JS, Noel J, Glass RI. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. J Infect Dis **2000**; 181 Suppl 2: S284-7.
130. Ahmed SM, Lopman BA, Levy K. A systematic review and meta-analysis of the global seasonality of norovirus. PLoS One **2013**; 8(10): e75922.
131. Lopman B, Armstrong B, Atchison C, Gray JJ. Host, weather and virological factors drive norovirus epidemiology: time-series analysis of laboratory surveillance data in England and Wales. PLoS One **2009**; 4(8): e6671.
132. Colas de la Noue A, Estienney M, Aho S, et al. Absolute Humidity Influences the Seasonal Persistence and Infectivity of Human Norovirus. Appl Environ Microbiol **2014**; 80(23): 7196-205.
133. Sukhrie FH, Siebenga JJ, Beersma MF, Koopmans M. Chronic shedders as reservoir for nosocomial transmission of norovirus. J Clin Microbiol **2010**; 48(11): 4303-5.
134. Makison Booth C. Vomiting Larry: a simulated vomiting system for assessing environmental contamination from projectile vomiting related to norovirus infection. J Infect Prev **2014**; 15(5): 176-80.
135. Makison Booth C, Frost G. Potential distribution of viable norovirus after simulated vomiting. J Hosp Infect **2019**; 102(3): 304-10.
136. Nenonen NP, Hannoun C, Svensson L, et al. Norovirus GII.4 detection in environmental samples from patient rooms during nosocomial outbreaks. J Clin Microbiol **2014**; 52(7): 2352-8.

137. Bonifait L, Charlebois R, Vimont A, et al. Detection and quantification of airborne norovirus during outbreaks in healthcare facilities. Clin Infect Dis **2015**; 61(3): 299-304.
138. Cheesbrough JS, Barkess-Jones L, Brown DW. Possible prolonged environmental survival of small round structured viruses. J Hosp Infect **1997**; 35(4): 325-6.
139. Seitz SR, Leon JS, Schwab KJ, et al. Norovirus infectivity in humans and persistence in water. Appl Environ Microbiol **2011**; 77(19): 6884-8.
140. Tung-Thompson G, Gentry-Shields J, Fraser A, Jaykus LA. Persistence of human norovirus RT-qPCR signals in simulated gastric fluid. Food Environ Virol **2015**; 7(1): 32-40.
141. Kampf G. Efficacy of ethanol against viruses in hand disinfection. J Hosp Infect **2018**; 98(4): 331-8.
142. Costantini V, Morantz EK, Browne H, et al. Human Norovirus Replication in Human Intestinal Enteroids as Model to Evaluate Virus Inactivation. Emerg Infect Dis **2018**; 24(8): 1453-64.
143. Cromeans T, Park GW, Costantini V, et al. Comprehensive comparison of cultivable norovirus surrogates in response to different inactivation and disinfection treatments. Appl Environ Microbiol **2014**; 80(18): 5743-51.
144. Bitler EJ, Matthews JE, Dickey BW, Eisenberg JN, Leon JS. Norovirus outbreaks: a systematic review of commonly implicated transmission routes and vehicles. Epidemiol Infect **2013**; 141(8): 1563-71.
145. Schmid D, Kuo HW, Hell M, et al. Foodborne gastroenteritis outbreak in an Austrian healthcare facility caused by asymptomatic, norovirus-excreting kitchen staff. J Hosp Infect **2011**; 77(3): 237-41.
146. Parron I, Alvarez J, Jane M, et al. A foodborne norovirus outbreak in a nursing home and spread to staff and their household contacts. Epidemiol Infect **2019**; 147: e225.
147. Barclay L, Park GW, Vega E, et al. Infection control for norovirus. Clin Microbiol Infect **2014**; 20(8): 731-40.
148. Lopman B, Gastanaduy P, Park GW, Hall AJ, Parashar UD, Vinje J. Environmental transmission of norovirus gastroenteritis. Curr Opin Virol **2012**; 2(1): 96-102.
149. Xie X, Li Y, Chwang AT, Ho PL, Seto WH. How far droplets can move in indoor environments--revisiting the Wells evaporation-falling curve. Indoor Air **2007**; 17(3): 211-25.
150. Gralton J, Tovey E, McLaws ML, Rawlinson WD. The role of particle size in aerosolised pathogen transmission: a review. J Infect **2011**; 62(1): 1-13.
151. Morawska L. Droplet fate in indoor environments, or can we prevent the spread of infection? Indoor Air **2006**; 16(5): 335-47.
152. Tung-Thompson G, Libera DA, Koch KL, de Los Reyes FL, 3rd, Jaykus LA. Aerosolization of a Human Norovirus Surrogate, Bacteriophage MS2, during Simulated Vomiting. PLoS One **2015**; 10(8): e0134277.
153. Nazaroff WW. Norovirus, gastroenteritis, and indoor environmental quality. Indoor Air **2011**; 21(5): 353-6.

154. Sawyer LA, Murphy JJ, Kaplan JE, et al. 25- to 30-nm virus particle associated with a hospital outbreak of acute gastroenteritis with evidence for airborne transmission. Am J Epidemiol **1988**; 127(6): 1261-71.
155. Chadwick PR, McCann R. Transmission of a small round structured virus by vomiting during a hospital outbreak of gastroenteritis. J Hosp Infect **1994**; 26(4): 251-9.
156. Chadwick PR, Walker M, Rees AE. Airborne transmission of a small round structured virus. Lancet **1994**; 343(8890): 171.
157. Marks PJ, Vipond IB, Carlisle D, Deakin D, Fey RE, Caul EO. Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant. Epidemiol Infect **2000**; 124(3): 481-7.
158. Marks PJ, Vipond IB, Regan FM, Wedgwood K, Fey RE, Caul EO. A school outbreak of Norwalk-like virus: evidence for airborne transmission. Epidemiol Infect **2003**; 131(1): 727-36.
159. Wikswo ME, Cortes J, Hall AJ, et al. Disease transmission and passenger behaviors during a high morbidity Norovirus outbreak on a cruise ship, January 2009. Clin Infect Dis **2011**; 52(9): 1116-22.
160. Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinje J. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol **2014**; 52(1): 147-55.
161. Bert F, Scaioli G, Gualano MR, et al. Norovirus outbreaks on commercial cruise ships: a systematic review and new targets for the public health agenda. Food Environ Virol **2014**; 6(2): 67-74.
162. Thornley CN, Emslie NA, Sprott TW, Greening GE, Rapana JP. Recurring norovirus transmission on an airplane. Clin Infect Dis **2011**; 53(6): 515-20.
163. Angelo KM, Nisler AL, Hall AJ, Brown LG, Gould LH. Epidemiology of restaurant-associated foodborne disease outbreaks, United States, 1998-2013. Epidemiol Infect **2017**; 145(3): 523-34.
164. Evans MR, Meldrum R, Lane W, et al. An outbreak of viral gastroenteritis following environmental contamination at a concert hall. Epidemiol Infect **2002**; 129(2): 355-60.
165. Guzman-Herrador B, Heier B, Osborg E, Nguyen V, Vold L. Outbreak of norovirus infection in a hotel in Oslo, Norway, January 2011. Euro Surveill **2011**; 16(30).
166. Raj P, Tay J, Ang LW, et al. A large common-source outbreak of norovirus gastroenteritis in a hotel in Singapore, 2012. Epidemiol Infect **2017**; 145(3): 535-44.
167. Lopes-Joao A, Mesquita JR, de Sousa R, et al. Country-wide surveillance of norovirus outbreaks in the Portuguese Army, 2015-2017. J R Army Med Corps **2018**; 164(6): 419-22.
168. Repp KK, Keene WE. A point-source norovirus outbreak caused by exposure to fomites. J Infect Dis **2012**; 205(11): 1639-41.
169. Becker KM, Moe CL, Southwick KL, MacCormack JN. Transmission of Norwalk virus during a football game. N Engl J Med **2000**; 343(17): 1223-7.

170. Lysen M, Thorhagen M, Brytting M, Hjertqvist M, Andersson Y, Hedlund KO. Genetic diversity among food-borne and waterborne norovirus strains causing outbreaks in Sweden. J Clin Microbiol **2009**; 47(8): 2411-8.
171. Verhoef L, Hewitt J, Barclay L, et al. Norovirus genotype profiles associated with foodborne transmission, 1999-2012. Emerg Infect Dis **2015**; 21(4): 592-9.
172. Franck KT, Nielsen RT, Holzknecht BJ, Ersboll AK, Fischer TK, Bottiger B. Norovirus Genotypes in Hospital Settings: Differences Between Nosocomial and Community-Acquired Infections. J Infect Dis **2015**; 212(6): 881-8.
173. Dingle KE, Norovirus Infection Control in Oxfordshire Communities H. Mutation in a Lordsdale norovirus epidemic strain as a potential indicator of transmission routes. J Clin Microbiol **2004**; 42(9): 3950-7.
174. Sukhrie FH, Beersma MF, Wong A, et al. Using molecular epidemiology to trace transmission of nosocomial norovirus infection. J Clin Microbiol **2011**; 49(2): 602-6.
175. Brown JR, Roy S, Shah D, et al. Norovirus Transmission Dynamics in a Pediatric Hospital Using Full Genome Sequences. Clin Infect Dis **2019**; 68(2): 222-8.
176. Holzknecht BJ, Franck KT, Nielsen RT, Bottiger B, Fischer TK, Fonager J. Sequence analysis of the capsid gene during a genotype II.4 dominated norovirus season in one university hospital: identification of possible transmission routes. PLoS One **2015**; 10(1): e0115331.
177. German RR, Lee LM, Horan JM, et al. Updated guidelines for evaluating public health surveillance systems: recommendations from the Guidelines Working Group. MMWR Recomm Rep **2001**; 50(RR-13): 1-35; quiz CE1-7.
178. Buehler JW, Hopkins RS, Overhage JM, Sosin DM, Tong V, Group CDCW. Framework for evaluating public health surveillance systems for early detection of outbreaks: recommendations from the CDC Working Group. MMWR Recomm Rep **2004**; 53(RR-5): 1-11.
179. Shah MP, Wikswo ME, Barclay L, et al. Near Real-Time Surveillance of U.S. Norovirus Outbreaks by the Norovirus Sentinel Testing and Tracking Network - United States, August 2009-July 2015. MMWR Morb Mortal Wkly Rep **2017**; 66(7): 185-9.
180. Hauri AM, Westbrock HJ, Claus H, et al. Electronic outbreak surveillance in Germany: a first evaluation for nosocomial norovirus outbreaks. PLoS One **2011**; 6(3): e17341.
181. Haller S, Eckmanns T, Benzler J, et al. Results from the first 12 months of the national surveillance of healthcare associated outbreaks in Germany, 2011/2012. PLoS One **2014**; 9(5): e98100.
182. Harris JP, Adams NL, Lopman BA, Allen DJ, Adak GK. The development of Web-based surveillance provides new insights into the burden of norovirus outbreaks in hospitals in England. Epidemiol Infect **2014**; 142(8): 1590-8.

183. Harris JP. Norovirus Surveillance: An Epidemiological Perspective. J Infect Dis **2016**; 213 Suppl 1: S8-S11.
184. Kaplan JE, Goodman RA, Schonberger LB, Lippy EC, Gary GW. Gastroenteritis due to Norwalk virus: an outbreak associated with a municipal water system. J Infect Dis **1982**; 146(2): 190-7.
185. Larsson C, Andersson Y, Allestam G, Lindqvist A, Nenonen N, Bergstedt O. Epidemiology and estimated costs of a large waterborne outbreak of norovirus infection in Sweden. Epidemiol Infect **2014**; 142(3): 592-600.
186. Riera-Montes M, Brus Sjolander K, Allestam G, Hallin E, Hedlund KO, Lofdahl M. Waterborne norovirus outbreak in a municipal drinking-water supply in Sweden. Epidemiol Infect **2011**; 139(12): 1928-35.
187. Sartorius B, Andersson Y, Velicko I, et al. Outbreak of norovirus in Vastra Gotaland associated with recreational activities at two lakes during August 2004. Scand J Infect Dis **2007**; 39(4): 323-31.
188. Hoebe CJ, Vennema H, de Roda Husman AM, van Duynhoven YT. Norovirus outbreak among primary schoolchildren who had played in a recreational water fountain. J Infect Dis **2004**; 189(4): 699-705.
189. Loury P, Le Guyader FS, Le Saux JC, Ambert-Balay K, Parrot P, Hubert B. A norovirus oyster-related outbreak in a nursing home in France, January 2012. Epidemiol Infect **2015**; 143(12): 2486-93.
190. Meghnath K, Hasselback P, McCormick R, et al. Outbreaks of Norovirus and Acute Gastroenteritis Associated with British Columbia Oysters, 2016-2017. Food Environ Virol **2019**; 11(2): 138-48.
191. Westrell T, Dusch V, Ethelberg S, et al. Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. Euro Surveill **2010**; 15(12).
192. Cook N, Williams L, D'Agostino M. Prevalence of Norovirus in produce sold at retail in the United Kingdom. Food Microbiol **2019**; 79: 85-9.
193. Hjertqvist M, Johansson A, Svensson N, et al. Four outbreaks of norovirus gastroenteritis after consuming raspberries, Sweden, June-August 2006. Euro Surveill **2006**; 11(9): E060907 1.
194. Muller L, Schultz AC, Fonager J, et al. Separate norovirus outbreaks linked to one source of imported frozen raspberries by molecular analysis, Denmark, 2010-2011. Epidemiol Infect **2015**; 143(11): 2299-307.
195. Sarvikivi E, Roivainen M, Maunula L, et al. Multiple norovirus outbreaks linked to imported frozen raspberries. Epidemiol Infect **2012**; 140(2): 260-7.
196. Tian P, Yang D, Mandrell R. Differences in the binding of human norovirus to and from romaine lettuce and raspberries by water and electrolyzed waters. J Food Prot **2011**; 74(8): 1364-9.
197. Hardstaff JL, Clough HE, Lutje V, et al. Foodborne and Food-Handler Norovirus Outbreaks: A Systematic Review. Foodborne Pathog Dis **2018**; 15(10): 589-97.
198. Franck KT, Lisby M, Fonager J, et al. Sources of Calicivirus contamination in foodborne outbreaks in Denmark, 2005-2011--the role of the asymptomatic food handler. J Infect Dis **2015**; 211(4): 563-70.

199. Espenhain L, Berg TC, Bentele H, Nygard K, Kacelnik O. Epidemiology and impact of norovirus outbreaks in Norwegian healthcare institutions, 2005-2018. J Hosp Infect **2019**.
200. Gaspard P, Ambert-Balay K, Mosnier A, et al. Burden of gastroenteritis outbreaks: specific epidemiology in a cohort of institutions caring for dependent people. J Hosp Infect **2015**; 91(1): 19-27.
201. Wikswo ME, Kambhampati A, Shioda K, et al. Outbreaks of Acute Gastroenteritis Transmitted by Person-to-Person Contact, Environmental Contamination, and Unknown Modes of Transmission--United States, 2009-2013. MMWR Surveill Summ **2015**; 64(12): 1-16.
202. Yen C, Wikswo ME, Lopman BA, Vinje J, Parashar UD, Hall AJ. Impact of an emergent norovirus variant in 2009 on norovirus outbreak activity in the United States. Clin Infect Dis **2011**; 53(6): 568-71.
203. Lopman BA, Reacher MH, Vipond IB, et al. Epidemiology and cost of nosocomial gastroenteritis, Avon, England, 2002-2003. Emerg Infect Dis **2004**; 10(10): 1827-34.
204. Kambhampati A, Koopmans M, Lopman BA. Burden of norovirus in healthcare facilities and strategies for outbreak control. J Hosp Infect **2015**; 89(4): 296-301.
205. Belliot G, Lopman BA, Ambert-Balay K, Pothier P. The burden of norovirus gastroenteritis: an important foodborne and healthcare-related infection. Clin Microbiol Infect **2014**; 20(8): 724-30.
206. Sandmann FG, Shallcross L, Adams N, et al. Estimating the Hospital Burden of Norovirus-Associated Gastroenteritis in England and Its Opportunity Costs for Nonadmitted Patients. Clin Infect Dis **2018**; 67(5): 693-700.
207. Danial J, Cepeda JA, Cameron F, Cloy K, Wishart D, Templeton KE. Epidemiology and costs associated with norovirus outbreaks in NHS Lothian, Scotland 2007-2009. J Hosp Infect **2011**; 79(4): 354-8.
208. Kanerva M, Maunula L, Lappalainen M, Mannonen L, von Bonsdorff CH, Anttila VJ. Prolonged norovirus outbreak in a Finnish tertiary care hospital caused by GII.4-2006b subvariants. J Hosp Infect **2009**; 71(3): 206-13.
209. Johnston CP, Qiu H, Ticehurst JR, et al. Outbreak management and implications of a nosocomial norovirus outbreak. Clin Infect Dis **2007**; 45(5): 534-40.
210. MacCannell T, Umscheid CA, Agarwal RK, et al. Guideline for the prevention and control of norovirus gastroenteritis outbreaks in healthcare settings. Infect Control Hosp Epidemiol **2011**; 32(10): 939-69.
211. Health Protection Agency, British Infection Association, Healthcare Infection Society, Infection Prevention Society, Confederation NCfHINHS. Guidelines for the management of norovirus outbreaks in acute and community health and social care settings. Available at: http://wwwhpaorguk/webc/HPAwebFile/HPAweb_C/1317131639453, **2012**.

212. Public Health Agency of Sweden. Vinterkräksjuka i vården. **2014**: 1-73.
213. Harris JP, Lopman BA, O'Brien SJ. Infection control measures for norovirus: a systematic review of outbreaks in semi-enclosed settings. J Hosp Infect **2010**; 74(1): 1-9.
214. Friesema IH, Vennema H, Heijne JC, et al. Norovirus outbreaks in nursing homes: the evaluation of infection control measures. Epidemiol Infect **2009**; 137(12): 1722-33.
215. Sadique Z, Lopman B, Cooper BS, Edmunds WJ. Cost-effectiveness of Ward Closure to Control Outbreaks of Norovirus Infection in United Kingdom National Health Service Hospitals. J Infect Dis **2016**; 213 Suppl 1: S19-26.
216. Lee BY, Wettstein ZS, McGlone SM, et al. Economic value of norovirus outbreak control measures in healthcare settings. Clin Microbiol Infect **2011**; 17(4): 640-6.
217. Harris JP, Adak GK, O'Brien SJ. To close or not to close? Analysis of 4 year's data from national surveillance of norovirus outbreaks in hospitals in England. BMJ Open **2014**; 4(1): e003919.
218. Illingworth E, Taborn E, Fielding D, Cheesbrough J, Diggle PJ, Orr D. Is closure of entire wards necessary to control norovirus outbreaks in hospital? Comparing the effectiveness of two infection control strategies. J Hosp Infect **2011**; 79(1): 32-7.
219. Haill CF, Newell P, Ford C, et al. Compartmentalization of wards to cohort symptomatic patients at the beginning and end of norovirus outbreaks. J Hosp Infect **2012**; 82(1): 30-5.
220. . WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care. Geneva, **2009**.
221. Park GW, Williamson KJ, DeBess E, et al. High Hand Contamination Rates During Norovirus Outbreaks in Long-Term Care Facilities. Infect Control Hosp Epidemiol **2018**; 39(2): 219-21.
222. Liu P, Yuen Y, Hsiao HM, Jaykus LA, Moe C. Effectiveness of liquid soap and hand sanitizer against Norwalk virus on contaminated hands. Appl Environ Microbiol **2010**; 76(2): 394-9.
223. Yeargin T, Buckley D, Fraser A, Jiang X. The survival and inactivation of enteric viruses on soft surfaces: A systematic review of the literature. Am J Infect Control **2016**; 44(11): 1365-73.
224. Morter S, Bennet G, Fish J, et al. Norovirus in the hospital setting: virus introduction and spread within the hospital environment. J Hosp Infect **2011**; 77(2): 106-12.
225. Gallimore CI, Taylor C, Gennery AR, et al. Environmental monitoring for gastroenteric viruses in a pediatric primary immunodeficiency unit. J Clin Microbiol **2006**; 44(2): 395-9.
226. Wu HM, Fornek M, Schwab KJ, et al. A norovirus outbreak at a long-term-care facility: the role of environmental surface contamination. Infect Control Hosp Epidemiol **2005**; 26(10): 802-10.

227. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, Clostridium difficile, and Acinetobacter species. Am J Infect Control **2010**; 38(5 Suppl 1): S25-33.
228. Gallimore CI, Taylor C, Gennery AR, et al. Contamination of the hospital environment with gastroenteric viruses: comparison of two pediatric wards over a winter season. J Clin Microbiol **2008**; 46(9): 3112-5.
229. Heijne JC, Teunis P, Morroy G, et al. Enhanced hygiene measures and norovirus transmission during an outbreak. Emerg Infect Dis **2009**; 15(1): 24-30.
230. Mitchell C, Meredith P, Richardson M, Greengross P, Smith GB. Reducing the number and impact of outbreaks of nosocomial viral gastroenteritis: time-series analysis of a multidimensional quality improvement initiative. BMJ Qual Saf **2016**; 25(6): 466-74.
231. Hei H, Bezpalko O, Smathers SA, Coffin SE, Sammons JS. Development of a novel prevention bundle for pediatric healthcare-associated viral infections. Infect Control Hosp Epidemiol **2018**; 39(9): 1086-92.
232. Bonten MJ, Slaughter S, Ambergen AW, et al. The role of "colonization pressure" in the spread of vancomycin-resistant enterococci: an important infection control variable. Arch Intern Med **1998**; 158(10): 1127-32.
233. Billgren M, Christenson B, Hedlund KO, Vinje J. Epidemiology of Norwalk-like human caliciviruses in hospital outbreaks of acute gastroenteritis in the Stockholm area in 1996. J Infect **2002**; 44(1): 26-32.
234. Lopman BA, Andrews N, Sarangi J, Vipond IB, Brown DW, Reacher MH. Institutional risk factors for outbreaks of nosocomial gastroenteritis: survival analysis of a cohort of hospital units in South-west England, 2002-2003. J Hosp Infect **2005**; 60(2): 135-43.
235. Mattner F, Mattner L, Borck HU, Gastmeier P. Evaluation of the impact of the source (patient versus staff) on nosocomial norovirus outbreak severity. Infect Control Hosp Epidemiol **2005**; 26(3): 268-72.
236. Korte S, Pettke A, Kossow A, Mellmann A, Willems S, Kipp F. Norovirus outbreak management: how much cohorting is necessary? J Hosp Infect **2016**; 92(3): 259-62.
237. Kampmeier S, Pillukat MH, Kossow A, Pettke A, Mellmann A. Infection of exposed patients during norovirus outbreaks: are there predictive parameters? J Hosp Infect **2017**; 96(1): 75-80.
238. Petrignani M, van Beek J, Borsboom G, Richardus JH, Koopmans M. Norovirus introduction routes into nursing homes and risk factors for spread: a systematic review and meta-analysis of observational studies. J Hosp Infect **2015**; 89(3): 163-78.
239. Mattner F, Guyot A, Henke-Gendo C. Analysis of norovirus outbreaks reveals the need for timely and extended microbiological testing. J Hosp Infect **2015**; 91(4): 332-7.

240. Beersma MF, Sukhrie FH, Bogerman J, et al. Unrecognized norovirus infections in health care institutions and their clinical impact. J Clin Microbiol **2012**; 50(9): 3040-5.
241. Harris JP, Lopman BA, Cooper BS, O'Brien SJ. Does spatial proximity drive norovirus transmission during outbreaks in hospitals? BMJ Open **2013**; 3(7).
242. Johnson DL, Mead KR, Lynch RA, Hirst DV. Lifting the lid on toilet plume aerosol: a literature review with suggestions for future research. Am J Infect Control **2013**; 41(3): 254-8.
243. Verani M, Bigazzi R, Carducci A. Viral contamination of aerosol and surfaces through toilet use in health care and other settings. Am J Infect Control **2014**; 42(7): 758-62.
244. Ciofi-Silva CL, Bruna CQM, Carmona RDC, et al. Norovirus Recovery from Floors and Air after Different Decontamination Protocols. J Hosp Infect **2019**.
245. Zelner J, Adams C, Havumaki J, Lopman B. Understanding the importance of contact heterogeneity and variable infectiousness in the dynamics of a large Norovirus outbreak. Clin Infect Dis **2019**.
246. Nguyen JL, Dockery DW. Daily indoor-to-outdoor temperature and humidity relationships: a sample across seasons and diverse climatic regions. Int J Biometeorol **2016**; 60(2): 221-9.
247. Wu YL, Yang XY, Ding XX, et al. Exposure to infected/colonized roommates and prior room occupants increases the risks of healthcare-associated infections with the same organism. J Hosp Infect **2018**.
248. Mitchell BG, Dancer SJ, Anderson M, Dehn E. Risk of organism acquisition from prior room occupants: a systematic review and meta-analysis. J Hosp Infect **2015**; 91(3): 211-7.
249. Weber DJ, Rutala WA, Anderson DJ, Chen LF, Sickbert-Bennett EE, Boyce JM. Effectiveness of ultraviolet devices and hydrogen peroxide systems for terminal room decontamination: Focus on clinical trials. Am J Infect Control **2016**; 44(5 Suppl): e77-84.
250. Anderson DJ, Chen LF, Weber DJ, et al. Enhanced terminal room disinfection and acquisition and infection caused by multidrug-resistant organisms and Clostridium difficile (the Benefits of Enhanced Terminal Room Disinfection study): a cluster-randomised, multicentre, crossover study. Lancet **2017**; 389(10071): 805-14.
251. Anderson DJ, Moehring RW, Weber DJ, et al. Effectiveness of targeted enhanced terminal room disinfection on hospital-wide acquisition and infection with multidrug-resistant organisms and Clostridium difficile: a secondary analysis of a multicentre cluster randomised controlled trial with crossover design (BETR Disinfection). Lancet Infect Dis **2018**; 18(8): 845-53.

252. Holmdahl T, Walder M, Uzcategui N, et al. Hydrogen Peroxide Vapor Decontamination in a Patient Room Using Feline Calicivirus and Murine Norovirus as Surrogate Markers for Human Norovirus. Infect Control Hosp Epidemiol **2016**; 37(5): 561-6.
253. Wallace RL, Ouellette M, Jean J. Effect of UV-C light or hydrogen peroxide wipes on the inactivation of methicillin-resistant Staphylococcus aureus, Clostridium difficile spores and norovirus surrogate. J Appl Microbiol **2019**; 127(2): 586-97.
254. Sukhrie FH, Teunis P, Vennema H, et al. Nosocomial transmission of norovirus is mainly caused by symptomatic cases. Clin Infect Dis **2012**; 54(7): 931-7.
255. Kirch W, Kirch W, SpringerLink. Encyclopedia of Public Health. Dordrecht: Springer Netherlands, **2008**.
256. Roy CJ, Milton DK. Airborne transmission of communicable infection--the elusive pathway. N Engl J Med **2004**; 350(17): 1710-2.
257. Jones RM, Brosseau LM. Aerosol transmission of infectious disease. J Occup Environ Med **2015**; 57(5): 501-8.
258. Herfst S, Bohringer M, Karo B, et al. Drivers of airborne human-to-human pathogen transmission. Curr Opin Virol **2017**; 22: 22-9.
259. Wei J, Li Y. Airborne spread of infectious agents in the indoor environment. Am J Infect Control **2016**; 44(9 Suppl): S102-8.
260. Uhrbrand K, Schultz AC, Koivisto AJ, Nielsen U, Madsen AM. Assessment of airborne bacteria and noroviruses in air emission from a new highly-advanced hospital wastewater treatment plant. Water Res **2017**; 112: 110-9.
261. Lei H, Li Y, Xiao S, et al. Routes of transmission of influenza A H1N1, SARS CoV, and norovirus in air cabin: Comparative analyses. Indoor Air **2018**; 28(3): 394-403.
262. Kraay ANM, Hayashi MAL, Hernandez-Ceron N, et al. Fomite-mediated transmission as a sufficient pathway: a comparative analysis across three viral pathogens. BMC Infect Dis **2018**; 18(1): 540.
263. Canales RA, Reynolds KA, Wilson AM, et al. Modeling the role of fomites in a norovirus outbreak. J Occup Environ Hyg **2019**; 16(1): 16-26.
264. Darley ESR, Vasant J, Leeming J, et al. Impact of moving to a new hospital build, with a high proportion of single rooms, on healthcare-associated infections and outbreaks. J Hosp Infect **2018**; 98(2): 191-3.

"Dr. Snow will say that the other evidence in proof of the propagation by water gives weight to his view of the case, but we may quite as well say that the evidence by transmission through the air is much more stringent, and gives weight to our hypothesis....

We have already said, that from the positive evidence adduced by Dr. Snow, we were unable to do more than conclude that he had rendered the transmission of cholera by water an hypothesis worthy of inquiry... We may be mistaken in this, and the evidence which seems weak to us may not be so to others.

Although we think that he is biased by his creed, and obstinately looks only in one direction, we close his book with the conviction that he is an honest and conscientious observer."

Edmund A Parkes, Review: Mode of communication of cholera by John Snow.
British and Foreign Medico-Churgical Review 1855;15:449–56.
Reprinted Int J Epidemiol 2013;42;1543–52.