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Elucidate the epidemiology and transmission of enteric viruses through genotype determination

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1. Background

Water is an important vector for pathogens transmitted faecally-orally as reviewed by Rodriguez et al. (2012). There are two main faecal contamination sources of surface and groundwater, *e.g.* municipal wastewater and manure. The former constitutes a main risk for transmission of enteric viruses in the environment since infected humans shed viruses in high amounts (Ozawa et al. 2007). In the water environment, non-enveloped enteric viruses are quite stable and the infectious dose after exposure is low (Teunis et al. 1999; Teunis et al. 2008). Another feature for many of them, especially for RNA viruses, is genetic variation and rapid genetic changes. Manure on the other hand is not considered to be as an important source of human enteric viruses in the environment, but it may enable transmission of zoonotic viruses.

Contaminated drinking water is one of the most efficient vehicles in terms of virus transmission to a large population. A waterborne outbreak caused by noroviruses (NoV) can usually be rapidly detected, since people of all ages become symptomatic within a couple of days. Based on genetic features, NoV are divided into six genogroups (I to VI), each of which contains numerous genotypes. Human NoV belong to genogroups I, II and IV. It is often possible to define the genotype even from a short sequence of virus capsid gene (or polymerase gene), which has been practical in investigations of NoV gastroenteritis outbreaks. Comparison of "fingerprint" nucleic acid sequences obtained from viruses in water and in humans has been helpful when searching for a contamination source. Based on NoV genotype data linked to foodborne outbreaks collected in a EU-project FBVE, it has been suggested that for outbreaks with particular source of contamination, typical genotype profiles can be found (Verhoef et al. 2010). In a recent study, Verhoef et al., (2015) have analyzed data in 1999-2015 and got result that 37% of foodborne NoV outbreaks are caused by mixture of GII.4 and other genotypes, 27% are caused by all other single genotypes and 10% are caused by GII.4.

Many other viruses, such as rotavirus (RoV) and sapoviruses (SaV), cause symptomatic infections mainly in children, and the transmission route via water, especially in case of a low level of contamination of water may not be easily recognized. SaV are divided into at least 5 genogroups (GI-GV), human viruses belong to GI, GII, GIV and GV and express numerous genotypes. RoV has complex classification system, since there are genotypes for each of the 11 RNA gene segments, the most common profiles being Wa-like, DS1-like and AU-like (Matthijnssens et al., 2008). Traditional classification is based on VP7 (G-types), VP4 (P-types) and VP6 (serogroup I and II). RoV of at least 11 G-types and 12 P-types infect humans.

Water can be behind foodborne outbreaks as well, for example in shellfish outbreaks, that are mainly caused by NoV and hepatitis A virus (HAV) that causes more serious disease. Recently, also frozen berries and dried tomatoes contaminated with HAV have caused disease outbreaks in European countries. HAV has 6 primate specific genotypes of which I, II and III infect humans. Human HAV are further divided in A and B classes.

As an emerging threat, the number of hepatitis E virus (HEV) cases has been increasing in many European countries. Traditionally, Europeans contracted illness with HEV while travelling in the tropical and subtropical countries, where genotype 1 and 2 were endemic. Nowadays, several European countries have reported domestic HEV cases, caused by genotype 3 or occasionally 4

viruses. Many of them may be zoonoses, since genotype 3 is prevalent in piglets and has also been detected in several other animal species. Transmission routes and contamination sources of HEV are, however, still relatively poorly known. Cross-species transmissions of other enteric viruses, NoV and RoV, have also been suggested. In case of adaptation to new host, RoV can disperse rapidly from continent to continent (Zeller et al., 2015).

In our study, the working hypotheses were that 1) molecular typing of virus isolates in clinical samples from waterborne outbreaks will provide an indication on whether some virus genotypes are more frequently waterborne than others and that 2) genetic characterization of virus isolates will determine the potential zoonotic origin of waterborne viruses. Here, we have collected data from 1) NoV outbreaks waterborne or linked to water in several northern and southern countries of Europe, as well as 2) information about the possible role of water in transmission of other enteric/hepatitis viruses, including those with potential for zoonotic transmission.

2. Material and methods

NoV outbreak data linked to water from WP2 participants (country/institute), Spain/**UB**, Denmark/ **DTU-FOOD**, Finland/**UH**, Portugal/**IST** and Sweden/**SLU** and**,** in case available to participants, was collected. The participants also provided data about possible waterborne transmission of zoonotic enteric viruses, including HEV, HAV, RoV, NoV and SaV. The data contained published data (both English and native languages), submissions, poster abstracts and personal data. The main focus was in the time period of 2009-2015. Publications with genotype information were preferred. Primers used for genotype identification of NoV at UB are shown in Figure 1. Primers used for genotype identification of HAV at UB are shown in Figure 2.

Primer name	Sequence	Location	Reference
COGIF	cgytggatgcgnttycatga	5291-5311[a]	Kageyama et al., 2003
G1SKF	ctgcccgaattygtaaatga	5342-5364[a]	Kojima et al. 2002
GISKR	ccaacccarccattrtaca	5653-5671[a]	Kojima et al. 2002
COG2F	cargarbcnatgttyagrtggatgag	5003- 5029[b]	Kageyama et al., 2003
G2SKF	cntgggagggcgatcgcaa	5046-5064[b]	Kojima et al. 2002
G2SKR	ccrccngcatrhccrttrtacat	5367-5389[b]	Kojima et al. 2002

a Reference strain Norwalk virus (NC_001959; M87661)
b Reference strain Lordsdale (X86557)

Figure 1. Norovirus typing primers (provided by UB).

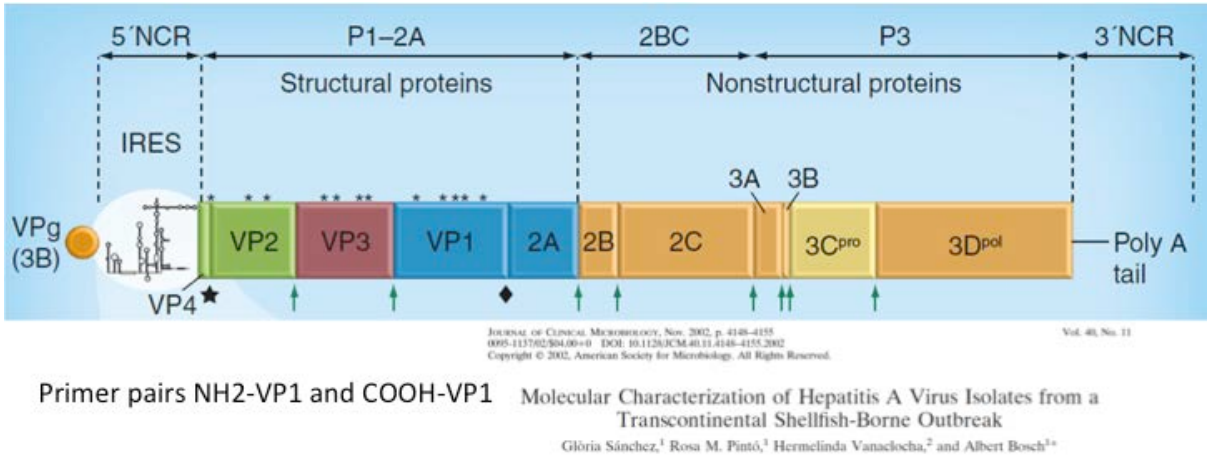

Figure 2. Hepatitis A virus typing primers (provided by UB).

3. Results

3.1. NoV genotypes linked to waterborne outbreaks

The NoV genotype range of in waterborne outbreaks reported in the publications reviewed here is broad, as can be seen in Table 1. The genotype GII.4 has been the causative agents in several outbreaks, but outbreaks caused by other GII strains, such as GII.6, GII.7 have also been reported. A large proportion of the outbreaks were caused by genogroup I viruses, such as GI.3, GI.4 and GI.7. Below there are more detailed descriptions of the outbreaks/studies reported by participant countries.

Spain

NoV in humans, Spain (Sabrià et al., 2014). Epidemiological work at the UB has enabled the study of the epidemiology of food and waterborne outbreaks of NoV gastroenteritis occurring in Catalonia during 2010-2012, and to compare clinical features and levels of viral shedding of the most prevalent GII.4 2012 variant with its predecessor. More than 50% of outbreaks were caused by genotype GII.4, although outbreaks caused by multiple strains, GII.6 and GII.1 were also prevalent. Clinical features of outbreaks caused by different genotypes circulating in Spain, including outbreaks caused by GII.4 2012 and GII.4 2009 strains, were comparable. Although shed at similar levels than GII.4 2009 strains, GII.4 2012 strains have clearly replaced the previous predominant strain.

Table 1. Publications describing NoV genotypes linked to water.

Publication	**Human/Water/ Sewage/Other**	**Virus**	**Genotypes**	**Country**
Perez-Sautu et al, 2012	River water	NoV	*GI.2, GI.3, GI.4, GI.5, GI.6, GI.7, GI.8, GI.NA2 and GI.NA3* *GII.3, GII.4, GII.6, GII.7, GII.13, GII.14 and GII.21*	ES
Sabria et al., 2014	Human	NoV	GI.3; GI.4; GI.7; GII.1; GII.2; GII.3; GII.4 (2009 and 2012); GII.5; **GII.6; GII.7;** GII.10; GII.12; GII.13; GII.16; GII.21	ES
Maunula et al., 2009	Human Municipal water	NoV (+AsV, RoV, AdV, EV) NoV RoV AdV	GI.4, GII.4-2006b, GII.7, GII.b **GII.4** G1P[8] AdV 40/41	FI
Maunula et al., 2012	Treated wastewater and river water	NoV	*GI, GII, (GII.4)*	FI
Maunula (2014)	Swimming water, in-lake beaches	NoV, (AdV)	**GI, GII**	FI
Soini et al. (submitted 2015	Human and well water	NoV	GI.3 (human) **GI** (water, typing unsuccessful)	FI
Müller et al., 2014	Human and Raspberries (probably sewage contaminated)	NoV	GI.b/I.6	DK
van Alphen et al., 2014	Human and drinking water contaminated with sewage water	NoV	**GII.4 New Orleans**	DK
(Nenonen, et al., 2012)	Human (following waterborne outbreak)	NoV SaV RoV AdV	GI.4; GI.7; GI.9; GI.3; GII.4; GII.6; GII.2 N.D. Type 2	SE
(Riera-Montes, et al., 2011)	Municipal water	NoV	GI.3	SE
(Hallin, 2012a)	Well	NoV	GI.4	SE
(Hallin, 2012a)	Municipal water	NoV	GII.4	SE
(Hallin, 2012a)	Well	NoV	GII.4 (human) **GI** (water)	SE
(Hallin, 2012a)	Well	NoV	GI.3	SE

(NoV genotypes identified from water samples linked to waterborne outbreaks are indicated in bold, if not linked to outbreaks, in italics.)

Viruses in river water in Spain (Perez-Sautu et al., 2012). A total of 147 samples (all NoV positive samples) were analysed for sequence determination. Seventyseven sequences were obtained from 50 samples, 44 belonging to GI and 33 belonging to GII. The occurrence of multiple genotypes was observed in all positive samples with the exception of freshwater samples collected at one site, located in the upstream part of the catchment and mainly in the winter period. Among the GI strains, nine different genotypes were detected (GI.2, GI.3, GI.4, GI.5, GI.6, GI.7, GI.8, GI.NA2 and GI.NA3), while seven genotypes (GII.3, GII.4, GII.6, GII.7, GII.13, GII.14 and GII.21) were detected belonging to GII. The genotypes GI.4 and GII.4 were highly prevalent. Several variants of genotype GII.4 were detected: 2002CN (three sequences), 2006a (one sequence), 2006b (four sequences) and two non-assigned sequences that were 99% identical to strain E3020 isolated in 2008 in Dijon, France.

Denmark

NoV in humans and berries (Müller et al., 2014): NoV outbreaks occur frequently in Denmark and it can be difficult to establish whether apparently independent outbreaks have the same origin. Here we report on six outbreaks linked to frozen raspberries, investigated separately over a period of 3 months. NoV from stools were sequence-typed; including extended sequencing of 1138 bp encompassing the hypervariable P2 region of the capsid gene. NoV was detected in 27 stool samples.

Genotyping showed genotype GI.Pb_GI.6 (polymerase/capsid) with 100% identical sequences. Samples from five outbreaks were furthermore identical over the variable capsid P2 region. In one outbreak at a hospital canteen, frozen raspberries was associated with illness by cohort investigation (relative risk 6·1, 95% confidence interval 3·2–11). Bags of raspberries suspected to be the source were positive for genogroup I and II NoVs, one typable virus was genotype GI.6 (capsid). These molecular investigations showed that the apparently independent outbreaks were the result of one contamination event of frozen raspberries. The contaminated raspberries originated from a single producer in Serbia and were originally not considered to belong to the same batch. The outbreaks led to consultations and mutual visits between producers, investigators and authorities. Further, Danish legislation was changed to make heat-treatment of frozen raspberries compulsory in professional catering establishments.

Waterborne outbreak in Denmark (van Alphen et al., 2014): In December 2012, an outbreak of acute gastrointestinal illness occurred in a geographical distinct area in Denmark covering 368 households. A combined microbiological, epidemiological and environmental investigation was initiated to understand the outbreak magnitude, pathogen(s) and vehicle in order to control the outbreak. NoV GII.4 New Orleans 2009 variant was detected in 15 of 17 individual stool samples from 14 households. NoV genomic material from water samples was detected and quantified and sequencing of longer parts of the viral capsid region (.1000 nt) was applied to patient and water samples. All five purposely selected water samples tested positive for NoV GII in levels up to 1.86104 genomic units per 200 ml. Identical NoV sequences were found in all 5 sequenced stool samples and 1 sequenced water sample, a second sequenced water sample showed 1 nt (,0.1%) difference. In a cohort study, including 256 participants, cases were defined as residents of the area experiencing diarrhoea or vomiting onset on 12–14 December 2012. We found an attack rate of 51%. Being a case

was associated with drinking tap-water on 12–13 December (relative risk = 6.0, 95%CI: 1.6–22) and a dose-response relation for the mean glasses of tap-water consumed was observed. Environmental investigations suggested contamination from a sewage pipe to the drinking water due to fall in pressure during water supply system renovations. The combined microbiological, epidemiological and environmental investigations strongly indicate the outbreak was caused by NoV contamination of the water supply system.

Finland

A large waterborne outbreak (Maunula et al., 2009, Laine et al., 2010). An inappropriate cross-connection between sewage and drinking water pipelines contaminated tap water in a Finnish town. About 450,000 l of treated sewage water was for 2 days allowed to run into the drinking water supplies of the city due to a personal error. An estimated 8453 residents fell ill during the outbreak (Laine et al., 2010). Several bacterial pathogens (*Campylobacter, Giardia*, non-typhoidal salmonella, *Clostridium difficile*) and viruses (see 3.2.) were found in patient samples and in drinking water. NoV were found in 29.8% of fecal samples; genotypes in humans were GI.4, GII.4-2006b, GII.7. GII.b. Water contained NoV GII.4.

Viruses in river water and wastewater, Finland (Maunula et al., 2012). The main objective of the study of was to determine the presence of human NoV in river water and in treated wastewater (TW) released into the river. During a one-year survey in 2007/2008, NoV was detected in 30.8% of river samples (20/65), and 40.5% of TW samples (17/45) with a real-time reverse transcription-PCR assay. NoV was present in the river water in the winter and spring, coinciding with the NoV epidemiological peak in the community and the presence of NoV in TW. Genogroup II NoV was detected more often than genogroup I NoV in river water. The presence of NoV genotype GII.4 could be determined in several river and TW samples when genotype GII.4 specific primers were used in a real-time RT-PCR. The continued monitoring in the spring of 2009 also revealed that the average concentration of NoV and adenoviruses (AdV) in TW was 2.64×10^3 and 1.29×10^4 pcr units per mL, respectively. No correlation between the presence of viruses and *Escherichia coli* was found.

NoV and bathers (Maunula, 2014). A cluster of gastroenteritis outbreaks caused by NoV occurred among bathers after they had visited in-lake beaches in July and August 2014 during several weeks of unusually warm temperature in Finland. In the end of July, there were several reports about gastroenteritis outbreaks both in northern and southern parts of the country. For example, over 1000 persons contracted illness in one city. NoV was detected in ill persons, many of which were children. Analyses of water taken from affected beaches in the metropolitan region revealed that in most cases water was of microbiologically good quality. Once NoV and once AdV was found in water of two swimming beaches. Contributing factors for the emergence of the outbreaks might have been overcrowdedness and limited water bodies. Epidemiological investigations are still ongoing. Never before gastroenteritis outbreaks among bathers with these dimensions have reported in Finland.

A waterborne outbreak at a hotel, well water. (Soini et al., 2015) In another case, it was found that the particular NoV GI.3 strain that caused a suspected waterborne gastroenteritis outbreak was not amplified efficiently with RT-qPCR assay, when a broadly-reacting primer-probe set was used. However, when the probe was omitted, improved amplification was observed. Thus, SYBRgreenI assay was applied for monitoring water and environmental samples. This way, NoV GI signal was

obtained in some well water samples. Also epidemiological analyses supported the option that drinking water was the source of this outbreak.

Sweden

Swedish waterborne outbreaks (Guzman-Herrador, et al., 2015), (Sartorius, et al., 2007), (Hallin, 2012b), (Riera-Montes et al. 2011), (Nenonen et al. 2012). Between 1998 and 2012, a total of 175 waterborne outbreaks affecting almost 86,000 individuals were notified in the Nordic countries. Most of these outbreaks were caused by viruses belonging to the *Caliciviridae* family. However most people got ill from *Cryptosporidium hominis* due to two large outbreaks in Sweden (Guzman-Herrador, et al., 2015). Further, a recreational water outbreak has been attributed to NoVs in Sweden (Data not shown; Sartorius, et al., 2007). The causes of some of the Swedish NoV drinking water outbreaks have been described by (Hallin, 2012b). Most of these are from smaller drinking water sources such as private wells and unchlorinated groundwater serving smaller municipalities. In these cases a single genotype can be the cause of the outbreak like during Easter 2009, when almost 200 people resident in a small Swedish village fell ill with gastrointestinal symptoms. A retrospective cohort study and a molecular investigation identified the source of the outbreak. Residents living in households connected to the public water network were at an increased risk of developing disease (relative risk 4.80, 95% confidence interval 1.68-13.73) compared to those with no connection to the public network. NoV genotype GI.3 was identified in stool samples from six patients and in a sample from the public water network. Contamination of one of the wells supplying the public water network was thought to be the source of the outbreak (Riera-Montes et al. 2011). On the other hand, in larger water systems where an outbreak has been caused by wastewater contamination from a larger city marked NoV diversity was detected in patient samples from a community outbreak of gastroenteritis with waterborne epidemiology affecting approximately 2,400 people (Nenonen et al. 2012). NoV was detected in 33 of 50 patient samples examined by group specific real-time reverse transcription-PCR. NoV genotype I (GI) strains predominated in 31 patients, with mixed GI infections occurring in 5 of these patients. Sequence analysis of RNA-dependent polymerase-N/S capsid-coding regions (900 nucleotides in length) confirmed the dominance of the GI strains (n36). Strains of NoV GI.4 (n21) and GI.7 (n9) were identified, but six strains required full capsid amino acid analyses (530 to 550 amino acids) based on control sequencing of cloned amplicons before the virus genotype could be determined. Three strains were assigned to a GI genotype, proposed as GI.9, based on capsid amino acid analyses showing 26% dissimilarity from the established genotypes GI.1 to GI.8. Three other strains grouped in a sub-branch of GI.3 with 13 to 15% amino acid dissimilarity to GI.3 GenBank reference strains. Phylogenetic analysis (2.1 kb) of 10 representative strains confirmed these genotype clusters. Strains of NoV GII.4 (n1), NoV GII.6 (n2), SaV GII.2 (n1, see also Table 2), RoV (n3), AdV (n1), and Campylobacter spp. (n2) were also detected as single infections or as mixtures with NoV GI (Nenonen et al. 2012).

3.2 Genotypes and transmission pathways for enteric viruses, including those with potential for zoonotic infections

While the role of NoVs in waterborne outbreaks has been clearly established, we know less about the transmission of other enteric viruses via water. Table 2 shows data for HEV, HAV, NoV, RoV and SaV, with focus in possible zoonotic transmission and connection to genotyping data, provided by the participants. Below are written detailed descriptions of the publications.

Spain

HAV in humans (D'Andrea et al., 2015). In a study focused on the effect of a universal hepatitis A vaccination program among preadolescents implemented in Catalonia, Spain, during the period of 1999–2013 its effectiveness was clearly demonstrated by an overall significant attack rate reduction. However, reductions were not constant over time, and increases were again observed in 2002–2009 due to the occurrence of huge outbreaks. In the following years, in the absence of large outbreaks, the attack rate decreased again to very low levels. However, an increase of symptomatic cases in the <5 age group has recently been observed. This is an unexpected observation since children younger than 6 are mostly asymptomatic. Such a long vaccination campaign offers the opportunity to analyze not only the effectiveness of vaccination, but also the influence of the circulating genotypes on the incidence of hepatitis A among the different age groups. This study has revealed the emergence of genotype IC during a foodborne outbreak, the short-lived circulation of vaccine-escape variants isolated during an outbreak among the men-having-sex-with-men group, and the association of genotype IIIA with the increase of symptomatic cases among the very young. From a public health perspective, two conclusions may be drawn: vaccination is better at an early age, and the vaccination schedule must be complete and include all recommended vaccine doses

SaV in river water, Spain (Sano et al., 2011). In this study, all human SaV genogroups (GI, GII, GIV, and GV) were detected. A total of 30 sequences were obtained, including 5 GI.1, 19 GI.2, 2 GII, 2 GIV, and 2 GV sequences. The most abundant genotype was GI.2, which was isolated from July 2008 to March 2009. Multiple genotypes were observed in some wastewater samples, and GI.2 was always the most prevalent sequence in these samples. On the clinical side, GI.2 has recently been detected quite frequently from gastroenteritis patients during a survey conducted in 2007 to 2009 in Netherlands, Sweden, Russia, and Slovenia, implying that GI.2 is widespread throughout Europe. Additionally, GI.2 shows a viral load in fecal samples that seems to be comparatively greater than that of other SaV types and possibly also shows increased virulence.

Denmark

HEV and rotavirus in blue mussels (Krog et al., 2014). Blue mussels (n=29) samples from commercial Danish harvesting areas were tested for HEV, RV-A, E. coli and Salmonella. While all samples were negative for HEV and RV-A, 8 and 13 samples were positive for E. coli (range 20-330 MPN/100 g shellfish flesh and liquid) or PCV2. A seasonal variation in PCV2 positive samples matched the periods for slurry application (positive during spring/fall and negative during summer). Bay and fjord areas were more likely to be PCV2 positive compared to open sea. E. coli does not correlate with PCV2, which we suggest should be further explored as a potential viral indicator for contamination of porcine waste.

Table 2. Studies revealing genotype data of enteric viruses, including those with zoonotic potential.

Publication	**Human/Animal/ Water/ Other**	**Virus**	**Genotypes**	**Country**
L. D'Andrea et al., 2015	Human (foodborne outbreak)	HAV	IC, IIIA, Vaccine-escape variant	ES
Sano et al., 2011	River water	SaV	5 GI.1, 19 GI.2, 2 GII, 2 GIV, and 2 GV	ES
Krog et al., 2014	Shellfish – animal virus	Porcine circovirus	PCV2	DK
Härö et al., 2014	Human (unknown sources, in one case juice made from contaminated well water)	SaV	GI.1, GII.1, GII.3, GII.5, GII.6 and GIV.1	FI
Jalava et al., 2014	Human (following waterborne outbreak)	SaV	GII.P3	FI
Maunula et al., 2009	Human Municipal water	(NoV, AsV, RoV, AdV, entero) (NoV) RoV AdV	GI.4 GII.4-2006b, GII.7, GII.b) (GII.4) G1P[8] AdV 40/41	FI
Maunula et al., 2013	Human	RoV	G10-P[14]-I2-R2-C2-M2-A3-N2-T6-E2-H3	FI
Kantala et al., 2009; Kantala et al., 2015	Human, Pigs	HEV	G1 (humans) G3e (pigs)	FI
Kettunen et al., 2013 (in Finnish)	Human (source unknown, but domestic case)	HEV	G3e	FI
(Nenonen, et al., 2012)	Human (following waterborne outbreak)	(NoV) SaV RoV AdV	GI.4; GI.7; GI.9; GI.3; GII.4; GII.6; GII.2 N.D. Type 2	SE
(Widen, et al., 2011)	Human, pig, wild boar	HEV	G1 (humans infected in Asia) G3 (Pigs, wild boars and humans infected in Europe)	SE
(Hellmer, et al., 2014)	Wastewater	HAV (NoV) HEV AsV RoV Aichi AdV	IB (GI and GII) N.D. N.D. N.D. N.D. N.D.	SE

Finland

SaV in water and in humans. **(Härö et al., 2014; Jalava et al, 2014).** At UH, archived human fecal samples negative for NoVs has been investigated for human SaV. The following SaV genotypes were detected in 18 SaV-positive stool suspensions from patients suffering from gastroenteritis: GI.1, GII.1, GII.3, GII.5, GII.6 and GIV.1 (Härö et al., 2014 poster abstract). SaV outbreaks have not been common in Finland, but a waterborne outbreak in 2012 was described (Jalava et al, 2014), in which SaV GII.P3 was detected in 5 out of 12 patient samples, some in adults. Although multiple viral and bacterial pathogens caused gastroenteritis in patients, they were not detected in the somewhat delayed water samples. However, according to epidemiological analysis, untreated drinking water was associated with illness.

RoV and SaV in a large waterborne outbreak (Maunula et al., 2009, Räsänen et al., 2010). In addition to bacterial pathogens and NoV (see 3.1.) the drinking water sample was positive for astroviruses (AsV), RoV, adenoviruses (AdV) and enteroviruses (EV). In another study (Räsänen et al., 2010) the following viruses were detected in hospitalized children after consuming water: NoV, SaV, Aichi virus, AdV, bocavirus. Mixed infections were common; as many as five viruses were found in one fecal sample from a child. Maunula et al. (2009) reported 30 double and 8 triple infections among the 294 patients. Fortunately, HAV was not detected in drinking water and no vaccination campaign was necessary to be implemented.

RoV, rare genotypes (Maunula et al., 2013). In this study rare RoV strains from Finnish children were characterized by sequence analysis. The RoV strain FI-436/2009 showed a rare Human Bovine -like genome profile of G10-P[14]-I2-R2-C2-M2-A3-N2-T6-E2-H3. The VP7 coding gene of FI-436/2009 had a nucleic acid identity of 98% with B75 bovine RoV.

HEV studies, pigs and human (Kantala et al., 2009; Kantala et al., 2015; Kettunen et al., 2013). In Finland, in a study where 105 sera from Finnish patients with acute hepatitis between 2000–2008 were investigated, 27.6% were found to contain anti-HEV IgM and/or IgG and 8.2% HEV RNA (Kantala et al., 2009). All HEV cases genotyped were G1, most had a travel history or no data was available. HEV genotype 3 has been found to be common among Finnish pigs (Kantala et al., 2015). Kettunen et al. (2013) described the first domestic HEV case in a previously healthy 53-year-old man without earlier travel history in Finland. The patient had serious symptoms, but recovered fully in three months. Serum was positive for anti-HEV IgM and for HEV RNA confirming the diagnosis of acute hepatitis E. The HEV was genotype 3 subtype e. When compared, the domestic human and porcine HEV 3e nucleic acid sequences available were closely related. Although they were not identical, the human virus differed from the Finnish porcine sequences as much as some of the porcine sequences differed from each other. No potential foodborne or other source of infection was found. Although the source of infection in this first verified human case of HEV genotype 3 in Finland remained unknown, zoonotic infection for example through domestic or imported pork or contaminated water directly or indirectly, cannot be ruled out.

Sweden

HEV study in humans, animals (Widén, *et al*., 2011). Partial HEV genomes from humans, pigs and wild boars were sequenced and compared by phylogeny showing close relatedness between HEV strains from humans with strains from pigs and wild boars from the same county. HEV strains form geographical clusters in the phylogenetic tree showing that there are endemic sources of HEV genotype 3 in Sweden. Sequencing of patient samples revealed that all 46 infected outside Europe were infected with genotype 1 whereas the HEV strains in sera from the 17 patients infected in Europe were all but one of genotype 3, the last one being infected by genotype 1 (Widén, *et al*., 2011). Further, deer have been found to be infected with G3 and G4 in Europe and Asia and have also been linked to human HEV infections (Tei et al. 2004). A study revealed high prevalence of HEV in Swedish moose. The genetic similarity of moose HEV to the zoonotic G3-4 belonging to species Orthohepevirus A may be indicative of a zoonotic potential also for the moose HEV (Lin et al. 2015). Improved typing methods are needed for the surveillance of potential animal reservoirs of zoonotic viruses and further to estimate the importance of these reservoirs to keep track of virus development.

HAV in wastewater (Hellmer et al, 2014). HAV was detected in wastewater in Gothenburg between weeks 5 and 13, 2013. Partial sequencing of the structural VP1protein identified three different strains of genotype IB. Two strains were involved in an ongoing (strawberry) outbreak in Scandinavia and were also identified in samples from patients with acute hepatitis A in Gothenburg during spring of 2013. The third strain was unique and was not detected in any patient sample (Hellmer, *et al*., 2014).

4. Conclusive results and discussion

Sewage of human origin contains all common enteric viruses and many of them can also be found in reduced numbers in river and seawater. This review also revealed that large outbreaks with high sewage contamination usually contained mixture of viruses and also multiple NoV genotypes (Maunula et al., 2009, Nenonen et al., 2012). In well water-related NoV outbreaks often only single NoV strain were found. Wells of small scale may be more commonly used in the North than South Europe and thus numerous northern water outbreaks linked to wells were reported. Systematic report of Matthews et al. (2012) revealed that NoV G1 was frequently linked to waterborne outbreaks. Although no systematic review process was performed here, G1 NoV was common in water, thus our results are in line with those of Matthews and others.

So far, at least north European countries do not have any evidence about domestic transmission of HEV G1 or G2, HEV cases with those genotypes were contracted after travelling. As expected, HEV G3 was prevalent in pigs of all participant countries and at least one zoonotic G3 HEV case was suggested. Direct waterborne HEV outbreaks were not reported. HEV G3 was not detected in shellfish in Denmark (Krog et al., 2014), so the seawater of the growing area in question was not contaminated with HEV.

Many species have their own NoV strains, but possible zoonoses have been searched for intensively over years. Human NoV genome has occasionally been detected in animinals, in a Norwegian rat (Wolf et al., 2013) and in pet dog feces (Summa et al., 2012). However, human sewage is still likely to pose the highest risk for transmission of human NoV and no evidence for the role of animals was obtained from the publications described in this review.

Like HEV and NoV, SaV infects pigs, but zoonotic infections in humans have not been reported; pigs and humans are infected by different genotypes. Possibility for cross-species contamination opens up in waterborne outbreaks, for example in a scenario where drinking water or growing area for shellfish is contaminated by porcine SaV. Primarily the presence of human SaV has been, indeed, reported in several waterborne outbreaks. SaV are frequently found in mixed infections in humans. In the publications presented here SaV was only one of the many contaminants found in waterborne outbreaks. SaV genotypes infecting humans were detected with no evidence for cross-species transmission.

Presence of RoV was reported in large waterborne outbreaks. RoV detected in these outbreaks were of human origin. One RoV strain isolated from a Finnish child showed a mosaic genome containing human and bovine RNA segments. Similar strains have been reported from other countries, so the zoonotic nature of the infection remains unclear. In Finland national vaccination of babies has efficiently decreased the number of RoV cases, but national RoV vaccination is not implemented in many European countries.

All the enteric viruses that have been discussed in this deliverable report can at least in theory contaminate food, such as berries and shellfish, through water. In addition to NoV, HAV is considered one of the most important foodborne viruses. The re-emergence of HAV outbreaks has been especially worrying in northern European countries, where no or a few foodborne HAV outbreaks appeared in 2000-2010 (depending on the country). According to this review, several different HAV types are currently circulating in Europe. Monitoring sewage for HAV proposed by Hellmer et al. (2014) could be helpful as an early warning system.

5. Conclusions

In conclusion, our data reveals a variety of strains in waterborne NoV outbreaks, hence there is a need for broad assays in primary detection for catching them. Genotyping is a critical tool in tracing contamination source in waterborne outbreaks caused by NoV, HAV and other enteric viruses. Although currently the other enteric viruses are present less frequently in waterborne outbreak situations, a threat of emerging, often zoonotic, waterborne pathogens cannot be excluded. In order to be prepared for such events, sensitive and specific genotyping methods must be available. Implementation of novel techniques, such as next generation sequencing, will offer more complete sequence data that can be used for virus strain comparisons in epidemiological investigations.

Our results, although descriptive and qualitative, give insight into how genetic characterization of viruses might reveal potential zoonotic origin of enteric viruses, such as in case of HEV and RoV. The data on animal and human virus genotype strains that have been collected for many years into databanks will provide a good basis in deducing the potential zoonotic origin of viruses in waterborne outbreaks. This information of virus genotypes together with source tracking analyses is valuable in determining the sources and hosts of water contamination.

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