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A household survey of intrafamily norovirus transmission

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ABSTRACT

Norovirus (NoV) is a leading cause of epidemic and sporadic gastroenteritis in people of all ages. Humans are the primary source of NoV and household contact is one of the risk factors for NoV transmission. However, the mechanisms underlying person-to-person NoV transmission are poorly understood. Here we conducted a survey to profile the frequency and characteristics of intrafamily NoV transmission. Stool samples were collected every week from three households between 2016–2020; the total number of samples was 1,105. The detection of NoV and the genotyping were performed by reverse transcription-polymerase chain reaction (RT-PCR) targeting the capsid region and direct sequencing methods. NoV was detected in 3.4% of all samples. Eight NoV genotypes were identified. The most common genotype was GII.17, followed in order by GII.6, GI.6, GII.4, GI.3, and GI.2/GI.8/GI.9. Most NoV-positive samples were obtained from asymptomatic individuals. The highest number of NoV transmissions was found in household 3 (6 infections), followed by household 2 (2 infections), while household 1 had no NoV transmission, suggesting that asymptomatic NoV carriers play a major role in infection as NoV reservoirs in the households. Further clarification of the mode of infection will contribute to improved understanding and an appropriate prevention.

(198 words)

1. INTRODUCTION

Norovirus (NoV) is a leading cause of epidemic and sporadic gastroenteritis in all age groups and geographical settings. Its genetic diversity and continuous evolution contribute to the persistence of NoV infections.[1] Pires et al.[2] estimated that the annual global NoV incidence was 677 million cases worldwide, with approximately 200,000 deaths in people at extremes of age. In Indonesia, the prevalence of NoV among children with acute gastroenteritis (AGE) was 15.4% - 18.5%.[3,4] In our previous study we observed that NoV was detected in 14 of the 512 stool samples (2.7%) obtained from 18 asymptomatic individuals. Interestingly, seven of the 18 individuals (38.9%) having at least 1 positive stool sample, with some asymptomatic individuals shed as much virus as symptomatic cases.[5]

NoV belongs to the family *Caliciviridae* and is composed of positive-sense, single-stranded RNA encased in a non-enveloped protein arrangement forming cup-like depressions. NoV is composed of 10 genogroups (GI–GX) and more than 48 genotypes, depending on VP1 sequences.[6] NoV is highly contagious and is transmitted primarily through direct contact with the vomit or faeces of patients or with faecally contaminated food or water, and subsequent contact with contaminated environmental surfaces.[7,8] NoV infection generally follows an incubation period of 12 to 48 h and is accompanied by

nausea, vomiting, watery diarrhea, and abdominal pain.[9, 10] Illness usually lasts only 2–3 days but may last longer (i.e. 4–6 days) in children younger than 11 years of age.[11,12] However, the virus can be shed at low titers for up to 8 weeks in previously healthy individuals, with a median of 28 days after inoculation.[13]

Humans are the primary source of NoV and household contact is one of the risk factors for NoV transmission.[14-16] Intrafamily transmission can occur because close contacts result in high exposure to the virus and secondary transmission. Although contact with symptomatic individuals has been considered the primary route of NoV transmission,[17] NoVs can also circulate in the home via asymptomatic infection,[18] which may suggest that young children are the main drivers of intrafamily NoV transmission.[19] Data on person-to-person NoV transmission is still limited and remains an area of great interest. In this study, we conducted a molecular epidemiological study of intrafamily NoV transmission in Surabaya, Indonesia, to profile the frequency of household transmission and its characteristics.

2. MATERIALS AND METHODS

2.1. Sample collection

We conducted a molecular epidemiological study of intrafamily NoV transmission

92 in Surabaya, Indonesia over 2016–2020. Three households of the following makeup
93 participated in this study:
94 - Household 1: mother (SF1) (29 y.o.), father (SF2) (34 y.o.), son (SF3) (3 y.o.) and
95 daughter (SF4) (2 y.o.) (from August 2016 to March 2018)
96 - Household 2: mother (AF1) (26 y.o.) and her first son (AF2) and second son (AF3) (4
97 y.o. and 3 y.o., respectively) (from February 2018 to November 2020)
98 - Household 3: mother (DF1) (29 y.o.), father (DF2) (29 y.o.), daughter (DF3) (5 y.o.)
99 and son (DF4) (2 y.o.) (from April 2018 to September 2019).
100 One or more stool samples were collected each week from each member of the three
101 households. All the stool samples were collected at the participant's home, stored at home
102 in a freezer at –20°C in new containers provided by the study team, and sent frozen to our
103 laboratory in the Institute of Tropical Disease, Universitas Airlangga in cool boxes. Stool
104 samples were sent along with the information on whether the person had diarrhea. For
105 each family, the mother provided informed consent for the collection and analysis of
106 samples. Sanitation and hygiene behavior and related medical history were also recorded.
107 This study was approved by the Research Ethics Board of both hospitals, Universitas
108 Airlangga (ethics approval number 2054/UN3.14/LT/2015) in Indonesia and Kobe
109 University (ethics approval number 1857) in Japan.

110

111 **2.2. NoV detection**

112 All collected samples were screened for the presence of NoV. First, 10% (w/v) of
113 the stool suspension of each sample was suspended in distilled water and centrifuged at
114 21,000 x g for 10 min. One μl of the supernatant from the 10% stool suspension was then
115 mixed with 19 μl of the sample treatment reagent from a One-step RT-PCR Norovirus G1
116 & G2 Detection Kit ver. 2 (Shimadzu, Kyoto, Japan), incubated at 90°C for 5 min and
117 stored on ice until further processing. RT-PCR was performed according to the
118 manufacturer's instructions. The amplified products specific to the target had an expected
119 size of 85 or 98 base pairs (bp) for G1 or G2, and the amplified product specific to the
120 internal control had an expected size of 251 bp. The supernatant was stored at –20°C until
121 further processing.

122 Samples that were determined to be positive using the Onestep RT-PCR Norovirus
123 G1 & G2 Detection Kit ver. 2 were then prepared for real-time PCR and conventional RT-
124 PCR and sequencing. Viral RNA was extracted from 140 μl of supernatant with a QIAamp
125 Viral RNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.
126 RNA was eluted with 60 μl of RNase- and DNase-free water and stored at −80 °C.

127 Genogroup-specific primers and probes were used to detect NoVs by TaqMan-

based real-time PCR using an ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA).[5] The primer and probe sets (COG1F/COG1R/RING1-TP(a)/RING1-TP(b)) and (COG2F/COG2R/ALPF/RING2AL-TP) were used to screen for GI and GII NoV strains, respectively. Real-time PCR for GI and GII were performed separately, including negative and positive controls in each experiment. Amplification was performed as described previously.[20, 21]

To confirm the NoV-positive results of the samples by real-time PCR, we performed a conventional RT-PCR and sequencing. The primer pair of G1SKF/G1SKR and G2SKF/G2SKR and the primer pair of G1F1/G1R1 and G2F1/G2R1 were used for detection of the capsid gene and genotyping of NoV GI and GII, respectively.[22]

2.3. Sequencing and phylogenetic analyses

The sequence of the NoV capsid gene was determined directly from the PCR product with a BigDye terminator cycle sequencing kit using a 3500XL Genetic Analyzer (Applied Biosystems, Foster, CA). Phylogenetic analyses were performed based on approximately 220–260 bp nucleic acid sequences of the amplified capsid region of GI and GII NoV. Reference sequences were retrieved from the DNA Data Bank of the Japan/European Molecular Biology Laboratory/GenBank database. Alignments were

146 performed using Bioedit software, and phylogenetic trees were constructed by the
147 neighbor-joining method. To confirm the reliability of the phylogenetic tree analysis,
148 bootstrap resampling and reconstruction were carried out 1000 times using Molecular
149 Evolutionary Genetic Analysis (MEGA X) software (http://www.megasoftware.net).
150
151 **3. RESULTS**
152 **3.1. Family characteristics**
153 Household 1 consisted of four people with different blood types (the father had type
154 O blood, the mother was type B blood, and the blood types of the children were unknown).
155 The father was the sole income provider; the mother worked as a homemaker and child
156 caretaker. The parents' educational background was strata 1. During this study, the son
157 was a kindergarten student. Refillable gallon-size jugs were used for drinking water, and
158 handwashing was done with soap and clean water stored in a bucket. The family members
159 rarely took anti-diarrheal medication, even if they had diarrhea (Table 1).
160 Household 2 consisted of four people. However, the father lived in a different area
161 and was not part of this study. The mother and the children had type O blood. Both parents
162 worked outside the home as income providers, and a nanny cared for the children when
163 both parents were at work. The parents' educational background was strata 1. During this

study, the sons were kindergarten students. Refillable gallon-size jugs were used for drinking water, and handwashing was done with soap and tap water. If a child had diarrhea, the mother administered lacto-B or Oralyte (Table 1).

Household 3 consisted of four people, all of whom had type B blood. Both parents worked as income providers, the father at a fast-food restaurant. When both parents were working, the children were cared for by their grandparents. The father's educational background was strata 1, while the mother's educational background was strata 2. During this study, the daughter was a kindergarten student. Prior to the start of this study, in February 2018, the son had been hospitalized for acute gastroenteritis and received a high dose of antibiotics and low-lactose milk. The mother reported that the son generally ate more than his sister and often engaged in mindless snacking. Refillable gallon-size jugs were used for drinking water, and handwashing was done with soap and tap water. The mother gave the children Pedialyte in the case of diarrhea (Table 1).

3.2. Detection of NoV strain transmission in the three households

NoV was detected in 3.4% (38/1,105) of the total samples from the three households. Of these 38 samples, four had cases of co-infection involving the GI and GII genotypes, all detected in household 3. Thus, GII (26/42, 62%) and/or GI (16/42, 38%) were

identified in 42 isolates (Fig. 1A and Table 4). The 42 strains comprised 8 NoV genotypes: GII.17 (n=11, 26.2%) was the most common, followed by GII.6 (n=9, 21.4%), GI.6 (n=8, 19.1%), GII.4 (n=6, 14.3%), GI.3 (n=5, 11.9%), and GI.2, GI.8, GI.9 (n=1, 2.4%, respectively) (Fig. 1B). The highest number of NoV-positive samples were obtained from the individuals without diarrhea, however the highest positivity was verified in individuals with diarrhea (6/52- 11.5%) when compared to those without diarrhea (32/1,053- 3.0%).

A total of 311 stool samples were collected from household 1 over a 20-month period (August 2016–March 2018) (Table 2). Diarrhea was reported only by the daughter, and she had diarrhea four times during the sampling period. There were 3 NoV-positive samples (0.97%), all in people without diarrhea. One NoV-positive sample (SF3 06022017) was collected from the son on February 2017 and was genotype GI.2. The other 2 samples were collected from the son (SF3 17012018) and his mother (SF1 31012018) over a two-week span (Table 2), and they were identified as different genotypes (GI.9 and GI.3, respectively) (Fig. 2). The 2 NoV-positive samples from the son had viral loads of 10^3–10^4 copies/g stool and the one of the mother was too low to be determined by real-time PCR (Table 2). Throughout the 20-month sample-collection period, there appears to have been no transmission of NoV in household 1.

A total of 455 stool samples were collected from household 2 over a 34-month period (February 2018–November 2020) (Table 3). During this period, the second son had diarrhea most often (11 times), followed by the mother (8 times) and the first son (6 times), but among the 6 NoV-positive samples (1.3%), none had diarrhea. The first 3 NoV-positive samples (AF3 16042018, AF2 12052018, AF2 18052018) were a consecutive series of samples obtained from the children two weeks apart over April and May 2018 (Table 3). All 3 of these samples were identified as GI.6 and they showed 100% sequence similarity (Fig. 2). A second series of 3 samples (AF3 08112020, AF3 11112020, AF2 14112020) were also obtained from the children during November 2020, and were identified as NoV GII.6 with high similarity (99.3–100%) (Fig. 3). Interestingly, the second son was always identified as NoV-positive first, followed by the first son. In both series of NoV-positive samples (in April-May 2018 and in November 2020), NoV transmission between children was confirmed (Table 3, Figs. 2-3). In the second series of 3 samples, collected in November 2020, the viral load of NoV-positive samples (10^3 copies/g stool) was higher than that (10^1–10^2 copies/g stool) in the first series collected in April 2018–May 2018 (Table 3).

A total of 339 stool samples were collected from household 3 over an 18-month period (April 2018–September 2019) (Table 4). This family experienced diarrhea (24/339,

7.1%) and NoV infection more frequently than the other households. During the sample-collection period, the mother and the father had diarrhea 3 and 5 times, respectively, while both the daughter and the son had diarrhea 8 times. NoV was detected in 27 (8.0%) samples, and 6 (22.2%) of these were collected from family members with diarrhea (3 of the 6 were from the son). NoV transmission in the family occurred 6 times, and a different genotype was identified each time. The first episode (April–May 2018), NoV was initially detected in the son, followed two weeks later by all three members. However, none of the family members reported having diarrhea (Table 4). All the NoV isolates (DF4 25042018, DF2 03052018, DF3 04052018, DF1 05052018, DF1 08052018) were identified as GI.6 with high sequence similarity (100%) (Fig. 2). These results suggest that NoV infection was transmitted from the son to the other three members of the family. The viral loads of NoV-positive samples were as low as only 10 copies/g stool. In the second episode, the son was again the first infected person (third week of May 2018), and a week later all three other members of the family were infected with the same strain (GII.17) with high sequence similarity (99.6–100%) (Table 4, Fig. 3). This infection continued and was last detected in the father in the third week of June 2018. The viral loads of the second NoV-positive samples were 10^1–10^2 copies/g stool, slightly higher than that in the first NoV-positive samples. In the third episode (second–third week of January 2019), NoV was

first detected in the daughter and a week later NoV was detected in the son. In the fourth episode (third–fourth week of January 2019), the mother was infected first, followed by the father (Table 4). In the third and the fourth episodes, all 6 NoV sequences were identified as GII.6, but they were separated into two clusters with 9% sequence difference (a cluster consisting of DF3 10012019 and DF4 20012019, and a cluster consisting of DF1 18012019, DF 1 26012019, DF2 26012019, and DF1 27012019) (Fig. 3). The third and fourth NoV-positive samples had higher viral loads (10^3 copies/g stool) than the previous ones. In the fifth episode (July–August 2019), NoV GII.4 was first detected in the son (high viral load, 10^5 copies/g stool), who was experiencing diarrhea, followed two weeks later by the daughter, and three weeks after that by the mother and the father in the second week of August 2019. Both parents were infected with both NoV GI.3 and NoV GII.4 (the fifth and sixth episode overlapped). In the sixth episode, the mother was infected with NoV GI.3 on August 5, 2019 and then the father and the daughter followed (Table 4). Those NoV GI.3 isolates (DF1 05082019, DF2 09082019, DF3 10082019, and DF1 26082019) showed high sequence similarity (99–100%) (Fig. 2). The viral load of these NoV-positive samples was 10^2 copies/g stool. In the fourth and fifth weeks of August 2019, NoV GII.4 was detected again, in the son followed by the mother (Table 4). All NoV GII.4 strains over July–August 2019 (DF4 12072019, DF3 2507019, DF1

05082019, DF2 09082019, DF4 21082019, and DF1 26082019) showed high sequence similarity (99.6–100%) (Fig. 3). In most of these episodes, the son was first infected with the different genotypes, followed by the other family members. These results suggest that an individual who is susceptible to NoV infection generally becomes infected first and then spreads the virus to other family members in the household. In addition, four cases of double NoV co-infection involving the GII and GI genotypes were detected, three in the mother, one with diarrhea (GII.4/GI.3) and two without diarrhea (GII.17/GI.8 and GII.4/GI.3), and one with diarrhea in the father (GII.4/GI.3).

4. DISCUSSION

In some countries that have introduced universal rotavirus vaccination, NoV is the leading cause of health care-acquired acute gastroenteritis in children.[23] However, rotavirus vaccination has not been implemented as a national immunization program in Indonesia. The low rotavirus vaccine coverage rate may be related to the higher prevalence of rotavirus (41–74.3%) than NoV (18.47–31.6%) in this country.[24-26]

In this prospective study conducted in Surabaya, Indonesia, three households with different lifestyles were enrolled. Samples were collected continuously for 18–34 months. Among the complete set of 1,105 samples, 3.4% (38/1,105) were NoV-positive,

and most of these were from asymptomatic individuals (3.0%, 32/1,053). This finding was consistent with a previous report in Surabaya[5] in which NoV was detected in 2.7% of stool samples from asymptomatic individuals. However, the positivity of NoV among individuals with diarrhea (6/52, 11.5%) was much higher than that among asymptomatic individuals (2.9%). The 6 samples were obtained from children (4 samples) and from parents (2 samples). In accordance with this result, the previous studies in Indonesia showed that the prevalence of NoV among children with AGE was 15.4% - 18.5%,[3,4] and that among adults with AGE was 9.1%.[27]

GII NoV strains were identified 1.6 times more frequently than GI strains. Previous studies have reported that GII NoV is the most prevalent worldwide[28], and the GII.4 genotype has predominated for the past two decades, causing both sporadic cases and outbreaks of NoV gastroenteritis.[29] Interestingly, we found eight genotypes, with GII.17 being the most common, followed by GII.6, GI.6, GII.4, GI.3, and GI.2/GI.8/GI.9 (Fig. 1B). Three of them (GI.6, GI.8 and GI.9) have never been reported in Indonesia before.

The viral load of GI NoV ranged from undetected to 10^4 copies/g stool, and that of GII NoV ranged from undetected to 10^5 copies/g stool (the highest viral load was detected in GII.4) (Tables 2-4). This is consistent with the results of the study by Ozawa et al.[30] In

290 that study, conducted in Japan during outbreaks and sporadic cases among food handlers,
291 NoV GII (especially GII.4) infections had slightly higher mean viral load than NoV GI
292 infections (especially GI.4).

293 In households 2 and 3, the duration of NoV shedding ranged from 1 week to 5 weeks
294 (with 1 week being most common) (Tables 3–4). There was a 1-week interval during May
295 2018 with undetected virus in the father and son in household 3 (Table 4). In a previous
296 report, an increase in viral load was observed on day 3 after the onset of NoV infection,
297 followed by a rapid decrease from day 10–15, but the load remained high for more than
298 a month in patients with severe combined immunodeficiency.[31] Moreover, in previously
299 healthy individuals, virus may be shed at low titers for up to 8 weeks.[13]

300 Among the three households in our study, there were differences in the rates of NoV
301 transmission. The highest number of NoV transmissions was 6 in household 3, followed
302 by 2 in household 2. Household 1 had no NoV transmission. A previous meta-analysis on
303 NoV confirmed the importance of person-to-person transmission and personal hygiene in
304 the faecal–oral transmission. Indirect transmission via the environment was also found to
305 play a role, including via untreated drinking water (in mixed populations), seafood
306 consumption (in mixed populations), and composite food (eating out/uncooked, in mixed
307 and young populations). [32] Another previous study showed several factors that contribute

to the high incidence of noroviruses among age groups and populations, including the amount of virus shed in the stool, low infectious dose, relative stability outside the human host, great viral diversity, and limited immunity.[33] All households in this study reported drinking safe water and having good handwashing practices, which may have been related to the higher levels of education among the parents. We speculate that one possible reason for the high rate of NoV infection in household 3 is that their son had been hospitalized for acute gastroenteritis and was given a large dose of antibiotics. He became ill in February 2018, just prior to his participation in this study in April 2018. He was first infected at a time when NoV infection was common in his family. It is likely that his underlying medical condition, such as disturbance of intestinal flora caused by use of antibiotics, made him susceptible to NoV infection and prone to diarrhea. The son's habit of "eating a lot" and "mindless snacking" may also have contributed to his NoV re-infections.

In addition, the son was infected with different NoV genotypes during this period, suggesting that he did not have a persistent infection. Genetic background, lifestyle, and living environment may affect the intestinal microbiota, making a person more susceptible to infection and more severe illness.[16] Some studies have investigated the association between histo-blood type antigens (HBGAs) and risk of NoV infection. A

meta-analysis suggested that the blood types A, B and AB may not affect susceptibility to NoV infection, but individuals with blood type O appear to be more susceptible.[34] On the other hand, a previous study in Africa reported that NoV infections were less common in children with the blood type A (OR 0.31; $P = 0.054$), GII.4 strains preferentially infected children with blood type B, and children with blood type O were infected with the largest variety of genotypes.[35] In the present study, household 3 (all of whose members were B blood type) were infected with multiple NoV genotypes, with GII.7 being the most common, and other NoV genotypes including GII.4 also being somewhat common, although the importance of blood types was unclear. Potential risk factors for NoV infection need to be further investigated.

The high similarity of NoV sequences obtained from families in two consecutive time periods in household 2 and six consecutive periods in household 3 suggests the occurrence of intrafamilial NoV infection. In addition, the two families were infected with various genotypes of NoV for short periods of time, mostly without inducing diarrheal symptoms. These results suggest that asymptomatic NoV carriers play an important role as NoV reservoirs in the households. Further analysis of the role of asymptomatic individuals in the community is necessary. In addition, transmission patterns and risk factors should be further investigated to provide appropriate recommendations for

prevention.

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Author Contributions

Juniastuti, Takako Utsumi, Maria Inge Lusida, Ikuo Shoji: designed the study and wrote the manuscript. Takako Utsumi, Zayyin Dinana, Laura Navika Yamani, Emily Gunawan: collected samples. Zayyin Dinana, Aussie Tahta Maharani, Anisa Lailatul Fitria, Rury Mega Wahyuni: carried out RVA detection and genome analysis. Takako Utsumi, Juniastuti: conducted sequence data analysis. Soetjipto, Yen Hai Doan, Hiroyuki Shimizu, Koji Ishii, Chieko Matsui, Lin Deng, Takayuki Abe, Kazuhiko Katayama, Maria Inge

Lusida, Ikuo Shoji: gave critical revision of the article. All the authors contributed to the interpretation of the data, writing of the manuscript, and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest regarding this study.

Data Availability Statement

The data that support the findings of this study will be available at Kernel-Kobe University Repository (https://da.lib.kobe-u.ac.jp/da/kernel/). The sequence results were deposited in the DDBJ/GenBank database with accession numbers LC764805-LC764820 and LC764769–LC764794.

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Figure legends

Figure 1. Percentage of NoV genogroups (A) and genotypes (B) obtained in this study. n indicates the amount of each genotype

Figure 2. Phylogenetic tree of the capsid region of GI NoV from members of all households. SF, AF, and DF are the samples from households 1, 2, and 3, respectively. SF1, 2, 3, and 4 are the mother, father, son, and daughter in household 1. AF1, 2, and 3 are the mother, son 1, and son 2 in household 2. DF1, 2, 3, and 4 are the mother, father, daughter, and son in household 3. The nucleotide sequences reported in this study were deposited in the DDBJ database under accession numbers LC764805-LC764820.

Figure 3. Phylogenetic tree of the capsid region of GII NoV from members of all households. SF, AF, and DF are the samples from households 1, 2, and 3, respectively. SF1, 2, 3, and 4 are the mother, father, son, and daughter in household 1. AF1, 2, and 3 are the mother, son 1, and son 2 in household 2. DF1, 2, 3, and 4 are the mother, father, daughter, and son in household 3. DF2 01062018–DF2 04062018 and DF1 26012019–DF1 27012019 were obtained in the same week, respectively. The nucleotide sequences reported in this study were deposited in the DDBJ database under accession numbers

500 LC764769-LC764794.

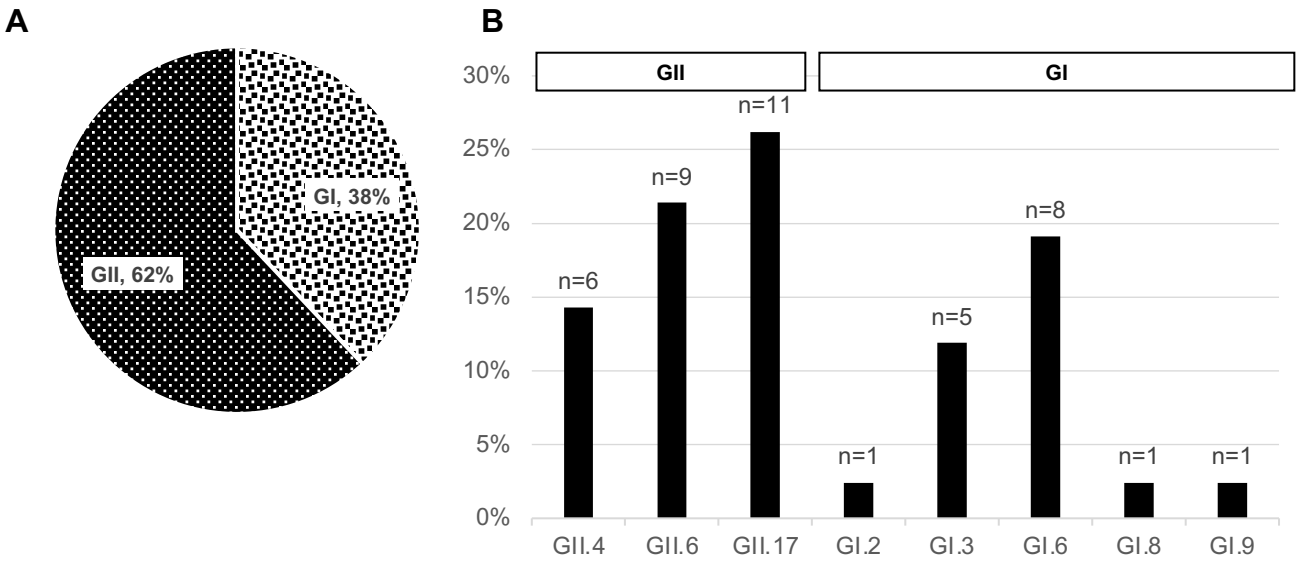

A
GI, 38%
GII, 62%
B
GII
GI
30%
25%
20%
15%
10%
5%
0%
n=6
n=9
n=11
n=1
n=5
n=8
n=1
n=1
GII.4
GII.6
GII.17
GI.2
GI.3
GI.6
GI.8
GI.9

Figure 1.

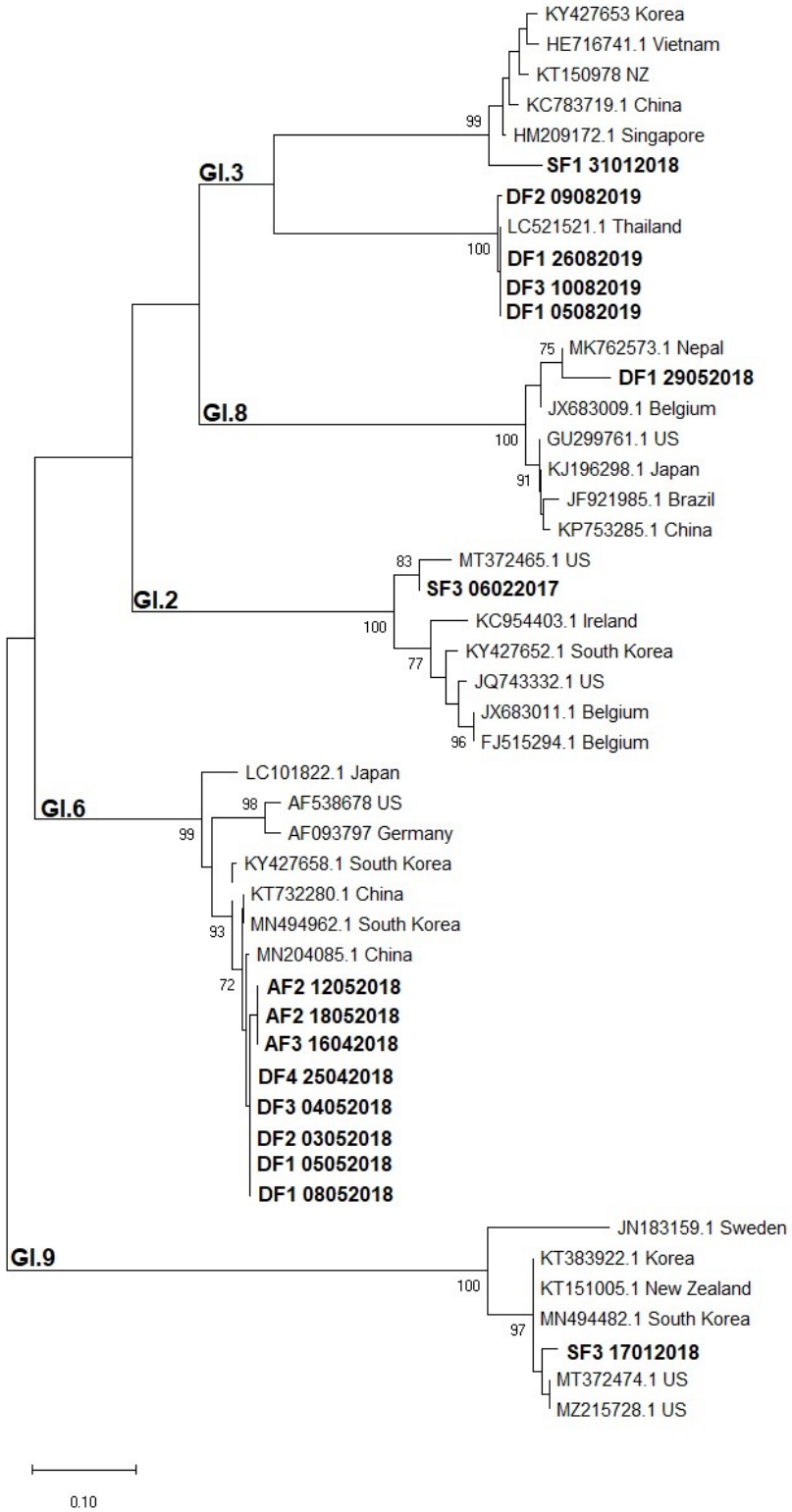

KY427653 Korea
HE716741.1 Vietnam
KT150978 NZ
KC783719.1 China
99
HM209172.1 Singapore
SF1 31012018
GI.3
DF2 09082019
LC521521.1 Thailand
100
DF1 26082019
DF3 10082019
DF1 05082019
75
MK762573.1 Nepal
DF1 29052018
GI.8
JX683009.1 Belgium
100
GU299761.1 US
KJ196298.1 Japan
91
JF921985.1 Brazil
KP753285.1 China
83
MT372465.1 US
SF3 06022017
GI.2
100
KC954403.1 Ireland
KY427652.1 South Korea
77
JQ743332.1 US
JX683011.1 Belgium
96
FJ515294.1 Belgium
LC101822.1 Japan
98
AF538678 US
GI.6
99
AF093797 Germany
KY427658.1 South Korea
KT732280.1 China
93
MN494962.1 South Korea
MN204085.1 China
72
AF2 12052018
AF2 18052018
AF3 16042018
DF4 25042018
DF3 04052018
DF2 03052018
DF1 05052018
DF1 08052018
JN183159.1 Sweden
GI.9
KT383922.1 Korea
100
KT151005.1 New Zealand
MN494482.1 South Korea
97
SF3 17012018
MT372474.1 US
MZ215728.1 US
0.10

Figure 2.

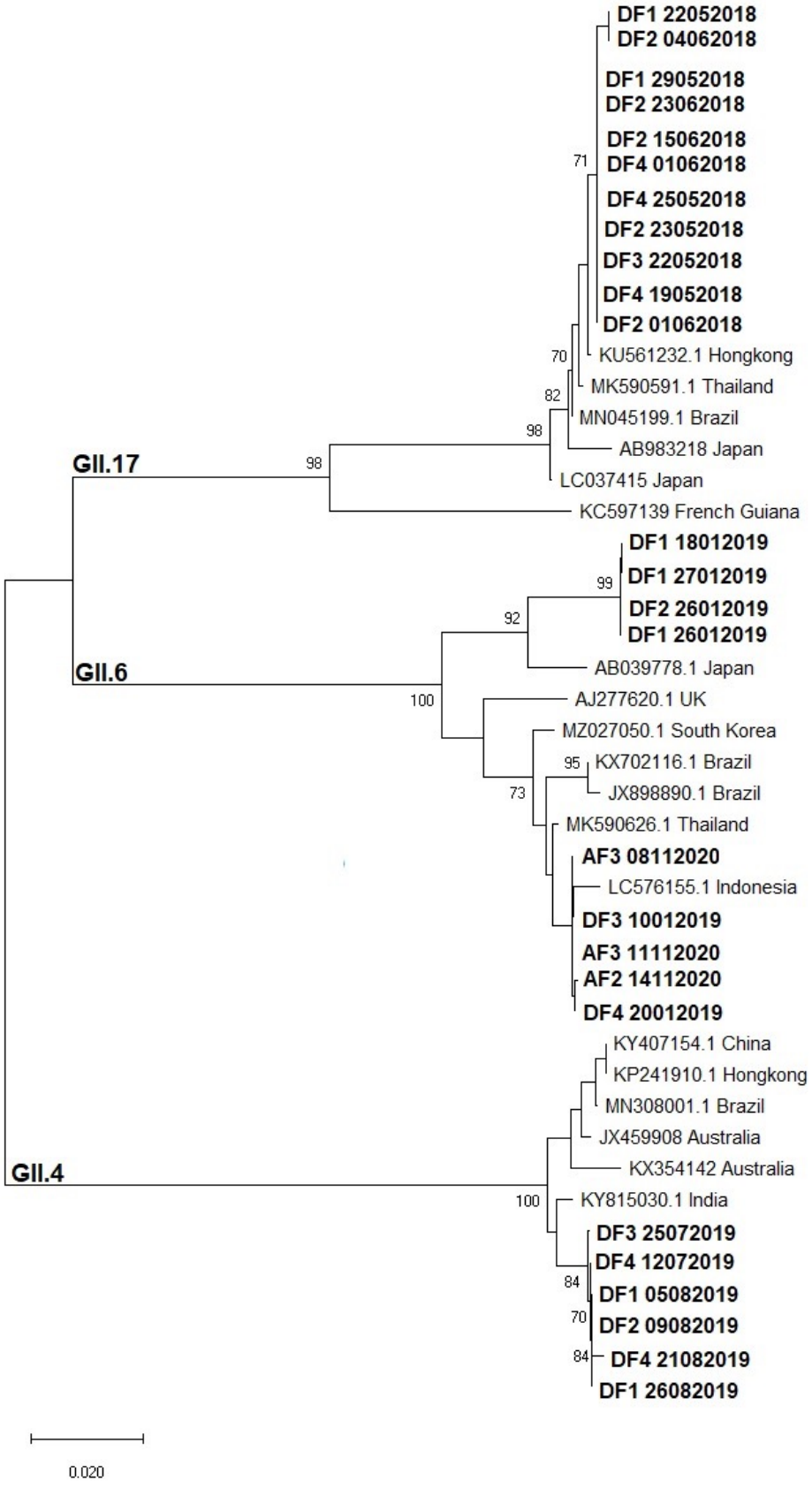

DF1 22052018
DF2 04062018
DF1 29052018
DF2 23062018
DF2 15062018
DF4 01062018
DF4 25052018
DF2 23052018
DF3 22052018
DF4 19052018
DF2 01062018
KU561232.1 Hongkong
MK590591.1 Thailand
MN045199.1 Brazil
AB983218 Japan
LC037415 Japan
KC597139 French Guiana
GII.17
DF1 18012019
DF1 27012019
DF2 26012019
DF1 26012019
AB039778.1 Japan
GII.6
AJ277620.1 UK
MZ027050.1 South Korea
KX702116.1 Brazil
JX898890.1 Brazil
MK590626.1 Thailand
AF3 08112020
LC576155.1 Indonesia
DF3 10012019
AF3 11112020
AF2 14112020
DF4 20012019
KY407154.1 China
KP241910.1 Hongkong
MN308001.1 Brazil
JX459908 Australia
KX354142 Australia
GII.4
KY815030.1 India
DF3 25072019
DF4 12072019
DF1 05082019
DF2 09082019
DF4 21082019
DF1 26082019
71
70
82
98
98
99
92
100
95
73
100
84
70
84
0.020

Figure 3.

Table 1. Characteristics of the three families

Characteristic	Family in household 1	Family in household 2	Family in household 3
Members	Mother (SF1) Father (SF2) Son (SF3) Daughter (SF4)	Mother (AF1) Father* Son 1 (AF2) Son 2 (AF3)	Mother (DF1) Father (DF2) Daughter (DF3) Son (DF4)
Blood type	Mother & father: O, Children: unknown	All members: O	All members: B
Breadwinner(s)	Father	Both parents	Both parents
Education level	Both parents: strata 1; son: kindergarten	Both parents: strata 1; children: kindergarten	Mother: strata 2; father: strata 1; daughter: kindergarten
Source of water for daily drinking	Refillable gallon-size water jugs	Refillable gallon-size water jugs	Refillable gallon-size water jugs
Handwashing	Soap and clean water in a bucket	Soap and tap water	Soap and tap water
Self-treatment for diarrhea	-	Lacto-B/oralyte	Pedialyte
Medical history	-	-	Son: Acute gastroenteritis

*The father of household 2 lived in a different area and was not a participant in this study.

Table 2. NoV genotypes, viral load and self-reported diarrhea in household 1 (August 2016–March 2018)

ID/Week	August 2016					September 2016					October 2016					November 2016					December 2016				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (SF1)																									
Father (SF2)																									
Son 1 (SF3)																									
Daughter 2 (SF4)																									

ID/Week	January 2017					February 2017					March 2017					April 2017					May 2017				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (SF1)																									
Father (SF2)																									
Son 1 (SF3)						¤ 10^3																			
Daughter 2 (SF4)																									

ID/Week	June 2017					July 2017					August 2017					September 2017					October 2017				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (SF1)																									
Father (SF2)																									
Son 1 (SF3)																									
Daughter 2 (SF4)																									

ID/Week	November 2017					December 2017					January 2018					February 2018					March 2018				
	I	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**
Mother (SF1)															♥UD										
Father (SF2)																									
Son 1 (SF3)														∇10^4											
Daughter 2 (SF4)																									

¤ = GI.2. ♥ = GI.3. ∇ = GI.9. Grey boxes = individuals with diarrhea; the number indicates the viral load (copies/gram). UD = undetected by rt-PCR.

Table 3. NoV genotypes, viral load and self-reported diarrhea in household 2 (February 2018 to November 2020)

ID/Week	February 2018					March 2018					April 2018					May 2018					June 2018				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (AF1)																									
Son 1 (AF2)																	♣10^1	♣10^2							
Son 2 (AF3)														♣10^1											

ID/Week	July 2018					August 2018					September 2018					October 2018					November 2018				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (AF1)																									
Son 1 (AF2)																									
Son 2 (AF3)																									

ID/Week	December 2018					January 2019					February 2019					March 2019					April 2019				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (AF1)																									
Son 1 (AF2)																									
Son 2 (AF3)																									

ID/Week	May 2019					June 2019					July 2019					August 2019					September 2019				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (AF1)																									
Son 1 (AF2)																									
Son 2 (AF3)																									

ID/Week	October 2019					November 2019					December 2019					January 2020					February 2020				
	I	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**
Mother (AF1)																									
Son 1 (AF2)																									
Son 2 (AF3)																									

ID/Week	March 2020					April 2020					May 2020					June 2020					July 2020				
	I	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**
Mother (AF1)																									
Son 1 (AF2)																									
Son 2 (AF3)																									

ID/Week	August 2020					September 2020					October 2020					November 2020				
	I	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**	**I**	**II**	**III**	**IV**	**V**
Mother (AF1)																				
Son 1 (AF2)																	♦10^3			
Son 2 (AF3)																♦10^3	♦10^3			

♣ = GI.6. ♦ = GII.6. Grey boxes = individuals with diarrhea; the number indicates the viral load (copies/gram).

Table 4. NoV genotypes, viral load and self-reported diarrhea in household 3 (April 2018 to September 2019)

ID/Week	April 2018			May 2018					June 2018					July 2018				
	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (DF1)				♣10^1	♣10^1		♠10^2	♠10^2 ▩10^1										
Father (DF2)				♣10^1			♠10^2		♠10^2	♠$10^{2\wedge}$	♠10^1							
Daughter 1 (DF3)				♣10^1			♠10^2											
Son 2 (DF4)		♣10^1				♠10^2	♠10^2		♠10^1									

ID/Week	August 2018					September 2018					October 2018					November 2018					December 2018				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (DF1)																									
Father (DF2)																									
Daughter 1 (DF3)																									
Son 2 (DF4)																									

ID/Week	January 2019					February 2019					March 2019					April 2019				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (DF1)			♦10^3	♦$10^{3\wedge}$																
Father (DF2)				♦10^3																
Daughter 1 (DF3)		♦10^3																		
Son 2 (DF4)			♦10^3																	

ID/Week	May 2019					June 2019					July 2019					August 2019				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Mother (DF1)																	• UD ♥10^2			•10^4 ♥10^2
Father (DF2)																	•10^3 ♥10^2			
Daughter 1 (DF3)														•10^3			♥10^2			
Son 2 (DF4)												•10^5							•10^2	

♣ = GI.6. ♠ = GII.17. ▒ = GI.8. ♦ = GII.6. • = GII.4. ♥ = GI.3. Grey boxes = individuals with diarrhea. ^ = 2 sequences were obtained on two dates in the same week. The 6 periods of transmission are encircled with the dotted line.