Studying the transmission dynamics of norovirus in a paediatric hospital

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Viral causes of diarrhoea

- Europe: 4 episodes of diarrhoea per child per year in under 5 year olds
- 147 community cases for every case reported in national surveillance
- 17 million community cases per year
- UK gastroenteritis: £115 million per year (63% norovirus)
- All faecal oral transmission (person-person)
## Norovirus

<table>
<thead>
<tr>
<th></th>
<th>Immunocompetent</th>
<th>Immunocompromised</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prevalence</strong></td>
<td>Leading worldwide cause of gastroenteritis</td>
<td>Not established (17-18%)&lt;sup&gt;1, 2&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Seasonality</strong></td>
<td>Winter peaks</td>
<td>Year-round&lt;sup&gt;2, 5&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
<td>Acute onset, vomiting (projectile, &lt;1 day), diarrhoea</td>
<td>Acute onset, vomiting (&lt;2 days), diarrhoea</td>
</tr>
<tr>
<td><strong>Duration</strong></td>
<td>24 – 48 hours</td>
<td>Weeks to years (chronic)</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td>Dehydration</td>
<td>Dehydration, malnutrition, dysfunction of intestinal barrier&lt;sup&gt;3&lt;/sup&gt;, dramatic weight loss&lt;sup&gt;4&lt;/sup&gt;, nutritional support</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td>Excellent</td>
<td>Poor to excellent (deaths&lt;sup&gt;3, 4&lt;/sup&gt;) Chronic infection common</td>
</tr>
</tbody>
</table>

<sup>1</sup>Schorn et al 2010; renal Tx; <sup>2</sup>Roddie et al. 2009 CID, HSCT; <sup>3</sup>Schwartz et al. 2011; <sup>4</sup>Roos-Weil et al. 2011, renal Tx; <sup>5</sup>Ludwig et al. 2008 cancer px
Norovirus genome & typing

Genogroup (GIi)  Genotype (GIi.4)  Variant type (GIi.4 Sydney 2012)
Norovirus genotypes identified in paediatric tertiary referral hospital (GOSH), July 2014 – February 2016 (n = 184)

>90% of outbreaks worldwide = GII.4
Norovirus capsid sequencing (1 kb)

Looks like 1 population
Norovirus Transmission Dynamics in a Pediatric Hospital Using Full Genome Sequences

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Background. Norovirus is a leading cause of worldwide and nosocomial gastroenteritis. The study aim was to assess the utility of molecular epidemiology using full genome sequences compared to routine infection prevention and control (IPC) investigations.

Can WGS be used to better understand the sources of norovirus infection and transmission dynamics in a paediatric population with a high prevalence of immunocompromised patients.

Conclusions. We show there are frequent introductions of multiple norovirus strains with extensive onward nosocomial transmission of norovirus in a pediatric hospital with a high proportion of immunosuppressed patients nursed in isolation. Phylogenetic analysis using full genome sequences is more sensitive than classic IPC investigations for identifying linked cases and should be considered when investigating norovirus nosocomial transmission. Sampling of staff, visitors, and the environment may be required for complete understanding of infection sources and transmission routes in patients with nosocomial infections not linked to other patients and among patients with phylogenetically linked cases but no evidence of direct contact.

Keywords. norovirus; epidemiology; molecular epidemiology; sequencing; whole genome.
Study Cohort

- Paediatric tertiary referral hospital, 350 beds, 60% single isolation rooms, no A & E

- Residual specimen (where available) from the first positive sample from all norovirus positive patients between 1st July 2014 and 17th February 2016 (19 months) was submitted for whole genome sequencing

- A total of 205 norovirus PCR positive patients were identified during the study period, 189 of these were whole genome sequenced

- Median patient age was 2 years

- 59% of patients profoundly immunocompromised
Infection Prevention and Control Decision tree following detection of Norovirus infection

- Norovirus PCR positive patient(s) and/or symptomatic staff or carers
  - Yes
    - Same ward?
      - No
        - No outbreak
      - Yes
        - Hospital acquired? (symptomatic / PCR + >48 hrs after admission)
          - No
            - No outbreak
          - Yes
            - Instigate control measures (see methods)
              - Same genogroup (GI / GII)?
                - No
                  - No outbreak
                - Yes
                  - ≥ 2 hospital acquired cases within 48-72 hr period
                    - Yes
                      - Suspected “IPC outbreak”
                        - Outbreak control meeting and actions
    - No outbreak

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Whole genome sequencing - SureSelect

Hybridize sample DNA to 120bp biotin-labelled RNA fragments to cover entire genome

Magnetic beads bind biotin-labelled DNA-RNA fragments

Pull out target DNA using magnet

Sample is now concentrated (not pre-amplified) and ready for sequencing

The child first and always
**Pros**

- Generates high yields of target DNA
- Sequence heterogeneity problematic for primer design therefore primers are often genotype specific.
- High failure rate, especially with non-target genotypes

**Cons**

- Overlapping PCR
- Direct sequencing of total RNA; no need for primer design
- No prior knowledge of sequences required
- Not genotype specific
- Majority of data is redundant with low proportion of reads generated corresponding to norovirus, resulting in low read depth and limited scope for variant analysis.

**Pros**

- Bait design accounts for sequence heterogeneity
- Good read depth, sufficient for variant analysis
- Successful with low titre samples

**Cons**

- Bait design relies on availability of sequences; novel genotypes that are highly divergent from known genotypes may have limited success
- Not genotype specific
Deep sequencing

Sanger sequencing
One consensus sequence for the sample
Cannot separate mixed sequences

Deep sequencing
A sequence for every piece of genomic material in the sample
1 sequence = 1 read
The child first and always

Norovirus full genome (7.5 kb)

Three distinct populations co-circulating within GII.4s
Sequence clusters identified by maximum likelihood phylogeny using full genome sequences.

<table>
<thead>
<tr>
<th>Sequence cluster number</th>
<th>Genotype</th>
<th>Number of patients</th>
<th>Date range</th>
<th>Number of wards</th>
<th>Number of clinical specialties involved</th>
<th>Bootstrap support</th>
<th>Diversity within cluster (SNPs)</th>
<th>Identified by infection control (IPC) investigations</th>
<th>Supported by classical epidemiology*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>GII.P7_GII.6</td>
<td>3</td>
<td>7 days</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>GII.P21_GII.3</td>
<td>17</td>
<td>3 months</td>
<td>6</td>
<td>3</td>
<td>100</td>
<td>0–22</td>
<td>Partially</td>
<td>Yes (16/17)</td>
</tr>
<tr>
<td>6</td>
<td>GII.P21_GII.3</td>
<td>2</td>
<td>3 days</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>GII.P21_GII.3</td>
<td>6</td>
<td>1 month</td>
<td>3</td>
<td>2</td>
<td>70</td>
<td>0–10</td>
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<tr>
<td>8</td>
<td>GII.P21_GII.3</td>
<td>2</td>
<td>2 months</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>GII.P21_GII.3</td>
<td>2</td>
<td>2 days</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>12</td>
<td>No</td>
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<td>10</td>
<td>GII.P21_GII.3</td>
<td>9</td>
<td>17 months</td>
<td>2</td>
<td>1</td>
<td>100</td>
<td>19–149</td>
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<td>Yes</td>
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<td>11</td>
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<td>2</td>
<td>3 months</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>29</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>12</td>
<td>GII.Pe_GII.4</td>
<td>8**</td>
<td>2 months</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>1–24</td>
<td>Partially</td>
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<tr>
<td>13</td>
<td>GII.Pe_GII.4</td>
<td>2</td>
<td>6 days</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>3</td>
<td>No</td>
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<tr>
<td>14</td>
<td>GII.Pe_GII.4</td>
<td>2</td>
<td>3 days</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
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<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>GII.Pe_GII.4</td>
<td>4</td>
<td>11 days</td>
<td>2</td>
<td>1</td>
<td>100</td>
<td>1–4</td>
<td>Partially</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>GII.P4_GII.4</td>
<td>3</td>
<td>3 days</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>1–3</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>17</td>
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<td>7</td>
<td>3 months</td>
<td>2</td>
<td>1</td>
<td>100</td>
<td>0–35</td>
<td>Partially</td>
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<tr>
<td>18</td>
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<td>2</td>
<td>25 days</td>
<td>2</td>
<td>1</td>
<td>100</td>
<td>14</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>19</td>
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<td>5</td>
<td>2.5 months</td>
<td>3</td>
<td>2</td>
<td>77</td>
<td>0–25</td>
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<td>Yes</td>
</tr>
<tr>
<td>20</td>
<td>GII.P4_GII.4</td>
<td>2</td>
<td>19 days</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>6</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>21</td>
<td>GII.P3_GII.3</td>
<td>2</td>
<td>8 months</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>31</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>22</td>
<td>GII.P2_GII.2</td>
<td>2</td>
<td>2 months</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>7</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>23</td>
<td>GII.P7_GII.6</td>
<td>2</td>
<td>3 months</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>17</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>24</td>
<td>GII.P21_GII.3</td>
<td>2</td>
<td>5.5 months</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>12</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>25</td>
<td>GII.P21_GII.3</td>
<td>3</td>
<td>4 months</td>
<td>3</td>
<td>1</td>
<td>95</td>
<td>17–28</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>26</td>
<td>GII.P21_GII.3</td>
<td>2</td>
<td>6 months</td>
<td>2</td>
<td>2</td>
<td>98</td>
<td>36</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>27</td>
<td>GII.P21_GII.3</td>
<td>2</td>
<td>3 months</td>
<td>2</td>
<td>1</td>
<td>100</td>
<td>18</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Maximum likelihood phylogeny of full genome sequences from norovirus episodes with GII.3 sequences

Cluster supported by classic epidemiology, but up to 149 SNPs between patients.

Immunocompromised, longer duration

Unsampled intermediates
Maximum likelihood phylogeny of full genome sequences from norovirus episodes with GII.4 sequences

Several clusters involve multiple wards
In many cases epi links were found (shared staff, equipment , use of common areas)
Relationship between clusters and wards in the hospital
Decision Tree based on combining Norovirus Sequencing and Epidemiological Data

Norovirus PCR positive patient(s) and/or symptomatic staff or carers

No outbreak

Monophyletic cluster on WGS?

Yes

Is there evidence for transmission?
- same ward and/or
- overlap in dates of hospital attendance either as in or outpatient and/or
- overlap in dates when norovirus PCR positive

No

Pairwise difference of ≤ 38 SNPs

GII.3/4

Yes

Epidemiologically Supported Cluster suggesting transmission occurred

No

Possibly not outbreak

Consider prolonged outbreak. Immunocompromised patients and intermediates

Epidemiologically unsupported Cluster
Conclusions

• Routine IPC investigations alone only identified linked transmission in 44% of cases compared with IPC and WGS

• In this study 33% of new norovirus cases were acquired from another patient, despite isolation nursing and stringent IPC measures

• Source of infection for 43% of nosocomial infections remains unknown even with WGS, wider sampling of patients, staff, visitors and the environment needed

• With ever decreasing sequencing costs and technologies that allow rapid turnaround times the possibility that norovirus genome sequencing could be used routinely to control nosocomial infections is now a reality
Acknowledgements

• Julianne Brown
• Judy Breuer
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• UCL Pathogen Genomics Unit (PGU)
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• UCL MRC CMMV.
• NIHR Great Ormond Street Hospital Biomedical Research Centre.

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