Noroviruses: Some Facts to Ponder Before Having Lunch!

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USDA-NIFA Food Virology Collaborative (NoroCORE)
Food Safety: a HUGE Issue!

“CDC estimates that each year roughly **1 in 6 Americans** (or 48 million people) gets sick, 128,000 are hospitalized, and 3,000 die of foodborne diseases…”

“Reducing foodborne illness by **10%** would keep about **5 million Americans** from getting sick each year.”

From: http://www.cdc.gov/foodborneburden/PDFs/FACTSHEET_A_FINDINGS_updated4-13.pdf
Two cost-of-illness models (basic and enhanced) with each accounting for health-related economic costs associated with foodborne illness

- Includes estimates of loss in productivity as well as actual treatment costs
- Enhanced model includes a measure for lost quality of life
- Costs calculated based on individual pathogens

Average cost per case of foodborne illness:

- Enhanced cost-of-illness model: $1,626 ($607 to $3,073)
- Basic model: $1,068 ($683 to $1,646)

Aggregated annual cost of illness:

- Enhanced cost-of-illness model: $77.7 billion ($28.6 to $144.6 billion)
- Basic model: $51.0 billion ($31.2 to $76.1 billion)
Most reported outbreaks and cases are caused by bacteria:

- Salmonella
- Campylobacter
- E. coli
- Cl. perfringens
- Shigella
- Staph. aureus

### Table 1: Number of reported foodborne-disease outbreaks, cases, and deaths, by etiology — United States, * 1993–1997*

<table>
<thead>
<tr>
<th>Etiology</th>
<th>Outbreaks</th>
<th>Cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>14 (0.5)</td>
<td>691 (0.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Brucella</td>
<td>1 (0.0)</td>
<td>19 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>25 (0.9)</td>
<td>539 (0.6)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>13 (0.5)</td>
<td>56 (0.1)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>57 (2.1)</td>
<td>2,772 (3.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>84 (3.1)</td>
<td>3,260 (3.8)</td>
<td>8 (27.6)</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>3 (0.1)</td>
<td>100 (0.1)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>357 (13.0)</td>
<td>32,610 (37.9)</td>
<td>13 (44.8)</td>
</tr>
<tr>
<td>Shigella</td>
<td>43 (1.6)</td>
<td>1,555 (1.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>42 (1.5)</td>
<td>1,413 (1.6)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Streptococcus, group A</td>
<td>1 (0.0)</td>
<td>122 (0.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Streptococcus, other</td>
<td>1 (0.0)</td>
<td>6 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>1 (0.0)</td>
<td>2 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>5 (0.2)</td>
<td>40 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>2 (0.1)</td>
<td>27 (0.0)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>Other bacterial</td>
<td>6 (0.2)</td>
<td>609 (0.7)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td><strong>Total bacterial</strong></td>
<td>655 (23.8)</td>
<td>43,821 (50.9)</td>
<td>28 (96.6)</td>
</tr>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciguatoxin</td>
<td>60 (2.2)</td>
<td>205 (0.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>4 (0.1)</td>
<td>17 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Monosodium glutamate</td>
<td>1 (0.0)</td>
<td>2 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Mushroom poisoning</td>
<td>7 (0.3)</td>
<td>21 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Scombrotokxin</td>
<td>69 (2.5)</td>
<td>297 (0.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Shellfish</td>
<td>1 (0.0)</td>
<td>3 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other chemical</td>
<td>6 (0.2)</td>
<td>31 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total chemical</strong></td>
<td>148 (5.4)</td>
<td>576 (0.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Parasitic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>4 (0.1)</td>
<td>45 (0.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Trichinella spiralis</td>
<td>2 (0.1)</td>
<td>19 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other parasitic</td>
<td>13 (0.5)</td>
<td>2,261 (2.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total parasitic</strong></td>
<td>19 (0.7)</td>
<td>2,325 (2.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Viral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>23 (0.8)</td>
<td>729 (0.8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Norwalk</td>
<td>9 (0.3)</td>
<td>1,233 (1.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Other viral</td>
<td>24 (0.9)</td>
<td>2,104 (2.4)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total viral</strong></td>
<td>56 (2.0)</td>
<td>4,066 (4.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Confirmed etiology</strong></td>
<td>878 (31.9)</td>
<td>50,788 (59.0)</td>
<td>28 (96.6)</td>
</tr>
<tr>
<td><strong>Unknown etiology</strong></td>
<td>1,873 (68.1)</td>
<td>35,270 (41.0)</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td><strong>Total 1993–1997</strong></td>
<td>2,751 (100.0)</td>
<td>86,058 (100.0)</td>
<td>29 (100.0)</td>
</tr>
</tbody>
</table>

*Includes Guam, Puerto Rico, and the U.S. Virgin Islands.
†Totals might vary by <1% from summed components because of rounding.
• Require a living host to multiply
• DNA or RNA inside a protein shell
• Very, very tiny
• Living???
  – Can't do anything without the host

Bacteria vs. Viruses

- Can multiply on their own
- DNA inside a membrane, with a more complex structure
- Ribosomes, flagella, etc.
- 10 to 100x bigger than viruses
- Living organisms
  - Take in energy, make wastes, multiply, move
Foodborne Disease: Etiologic Agents, 2006 – 2010

- HuNoV are the most common cause of foodborne illness

- Over 5.5 million cases of all foodborne illnesses each year are caused by human noroviruses (Scallan et al., 2011)

- May be a significant cause of foodborne disease of unknown etiology
Human Noroviruses: What’s the big deal?

New Norovirus Strain Rips Through The U.S.

by SCOTT HENSLEY
January 25, 2013 12:10 PM

Possible norovirus outbreak sicks dozens at Lafayette elementary school

NC sees increase in norovirus outbreaks

CHAPEL HILL, N.C. — Health departments across North Carolina have reported norovirus outbreaks in recent weeks, prompting state public health officials to issue an alert Tuesday.

The state Division of Public Health doesn’t track norovirus, so officials don’t have specific numbers of people sickened by the gastro-intestinal bugs. They said, however, that eight
Human Noroviruses (HuNoV) are the leading cause of acute gastroenteritis in all age groups in the United States (CDC estimates):  

- 21 million cases,  
- 70,000 hospitalizations, and  
- 800 deaths annually  
- $2 billion annually in healthcare and lost productivity costs
Norovirus in the U.S.

- 20 million Americans will get norovirus this year
  - 1 in 15 people

- About 365 foodborne norovirus outbreaks happen each year

- Most outbreaks happen in the winter (but this is changing...)
Discovery of the Norwalk Agent

- “Winter vomiting disease” in 1920’s
- Human challenge studies (1940s and 1950s) sought to identify causative agent of “acute non-bacterial gastroenteritis”
- In 1971, electron microscopic examination stool specimen collected from 1968 outbreak in Norwalk, Ohio revealed presence of 27 nm particle
- Subsequent human challenge studies confirmed this as the infectious agent

Miserable Symptoms

“The Norovirus: A Study in Puked Perfection” by Carl Zimmer

Today, The Guardian relayed one of those stunning medical stories that causes me to clean off my glasses and take another look to make sure I’m reading it clearly. They report that an outbreak of norovirus in Britain this winter has struck more than 1.1 million people with vomiting and diarrhea.

That’s right: 1.1 million. In Britain alone.

“Within a day of infection, noroviruses have rewired our digestive system so that stuff comes flying out from both ends” – Carl Zimmer in a recent National Geographic article.

– Vomiting, watery diarrhea, nausea, and abdominal pain.
– Usually self limiting, but in some instances (individuals with weak immune systems), complications from dehydration can develop.
Mode of Transmission in HuNoV Outbreaks, 20 States, 2009 (N=613)

- Person-to-person: 78%
- Foodborne: 15%
- Environmental: 0.2%
- Other/Unknown: 7%
- Waterborne: <0.1%
Foods Implicated* in Norovirus Outbreaks Reported to CDC by Commodity and Point of Contamination, 2001-2008

*Limited to outbreaks with a simple food (consisting of a single commodity) implicated
**Insufficient or conflicting information provided in outbreak report
Foods associated with norovirus outbreaks:

- Leafy greens
- Berries
- Molluscan shellfish
- Ready-to-eat foods
- Foods prepared by hand without a cooking step or handled after cooking
What Has the Last Two Decades Revealed?

- Member of *Caliciviridae* family (4 genera)
- Norovirus genogroups and genotypes
- Genetically and antigenically diverse
- Propensity toward mutation and recombination
  - Results in great strain diversity and frequent emergence of new epidemic strains

Genome organization and Capsid Structure

- Non enveloped; simple capsid structure
- Positive sense single-stranded RNA genome, 7.5 kb
- Protruding domain of capsid sequence important for receptor binding

A Challenge to Researchers

- No cell culture or animal model for cultivation (rely on RT-PCR)
- **Adequacy of cultivable surrogates**

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**Surrogates for the Study of Norovirus Stability and Inactivation in the Environment: A Comparison of Murine Norovirus and Feline Calicivirus**

JENNIFER L. CANNON,1 EFSTATHIA PAPAFRAGKOU,2 GEUNWOO W. PARK,1 JASON OSBORNE,3 LEE-ANN JAYKUS,2 AND JAN VINJÉ1*

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**pH stability for 30 min incubation**

Infectivity reduction (log PFU/ml)

MNV
FCV

A Challenge to Researchers: The “Infectivity Dilemma”

• What constitutes a “positive” for infectious virus?
  • Naked RNA vs. infectious virus
  • Particle:infectious particle ratio
  • Viral aggregation?
  • Gradual vs. instantaneous inactivation?
• Can we use RT-qPCR to measure virus infectivity?
  • Measuring capsid integrity
  • Measuring virion integrity
  • Likely to be process-specific
• Goal: positive RT-qPCR signal = Infectious particle
Are human noroviruses the perfect pathogen?

Characteristics of a "Perfect" Pathogen

<table>
<thead>
<tr>
<th>Considerations:</th>
<th>HuNoV:</th>
</tr>
</thead>
<tbody>
<tr>
<td>– Infectivity</td>
<td>– Highly contagious</td>
</tr>
<tr>
<td>– Transmissibility</td>
<td>– Rapid and efficient spread</td>
</tr>
<tr>
<td>– Environmental stability</td>
<td>– Environmentally stable and resistant to many sanitizers</td>
</tr>
<tr>
<td>– Evolutionary aspects</td>
<td>– Constantly evolving</td>
</tr>
<tr>
<td>– Immune response</td>
<td>– Evokes limited and likely short-lived immune response</td>
</tr>
<tr>
<td>– Morbidity and mortality</td>
<td>– Rarely lethal</td>
</tr>
</tbody>
</table>
HuNoV Infectivity

- Low infectious dose (≥18 viral particles)
- Copious shedding ($10^5$–$10^{11}$ viral copies per gram of feces), even among asymptomatic infections

Asymptomatic

Vomiting/diarhea

HuNoV environmental stability

- Environmentally stable
- Can persist on surfaces for up to 2 weeks or longer
HuNoV Stability and Infectivity Influence Indirect Transmission

Illustration of the direct and indirect transmission potential of norovirus over time.

- Direct transmission
- Environmentally-mediated transmission
- Vomiting incident

Day 1 | Days | One to two weeks

Time
HuNoV GII.4 persistence in suspension of SGF or PBS, over the course of 42 days, with or without RNase pretreatment prior to RT-qPCR
HuNoV Resistance—Surface Disinfectants

Tung et al. 2013, J. Food Prot. 76:1210-1217.
HuNoV Resistance—Hand Sanitizers

- Resistant to many common chemical disinfectants
  - notably resistant to ethanol based hand sanitizers

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**Effectiveness of Liquid Soap and Hand Sanitizer against Norwalk Virus on Contaminated Hands**

Pengbo Liu, Yvonne Yuen, Hui-Mien Hsiao, Lee-Ann Jaykus, and Christine Moe

Center for Global Safe Water, Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia 30322, and Department of Food Science, North Carolina State University, Raleigh, North Carolina 27695

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**TABLE 1. In vivo efficacies of hand wash agents against NV evaluated by standard and modified ASTM methods**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Standard ASTM method</th>
<th>Modified ASTM method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg log reduction (SD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No RNase</td>
<td>RNase</td>
</tr>
<tr>
<td>Dry control</td>
<td>0.20 (0.28)</td>
<td>0.16 (0.06)</td>
</tr>
<tr>
<td>Hand sanitizer</td>
<td>0.14 (0.31)</td>
<td>0.27 (0.12)</td>
</tr>
<tr>
<td>Liquid soap</td>
<td>0.94 (0.46)</td>
<td>0.67 (0.47)</td>
</tr>
<tr>
<td>Water rinse</td>
<td>0.75 (0.63)</td>
<td>0.58 (0.37)</td>
</tr>
</tbody>
</table>

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*Different superscript capital letter designations in a column indicate statistically significant differences (P < 0.05) between mean log10 reductions by disinfection/removal treatments for the finger pad eluates with no RNase treatment (A and B) or with RNase treatment (X and Y) prior to RT-qPCR.

*There was a marginal statistical difference (P = 0.048) between the results from the standard and modified ASTM methods for samples that received the RNase treatment.

*In comparing the results of the standard and modified ASTM methods for each disinfection/removal treatment, statistically significant differences (P < 0.05) were observed for treatments either with no RNase treatment or with RNase treatment prior to RT-qPCR.

Randomized, Double-Blinded Clinical Trial for Human Norovirus Inactivation in Oysters by High Hydrostatic Pressure Processing

Juan S. Leon,1,‡ David H. Kingsley,2‡ Julia S. Montes,1‡ Gary P. Richards,2 G. Marshall Lyon,3 Gwen M. Abdulhafid,3 Scot R. Seitz,3 Marina L. Fernandez,1 Peter F. Teunis,1 George J. Flick,4 and Christine L. Moe1*

Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia 30322; United States Department of Agriculture, Agricultural Research Service, Delaware State University, Dover, Delaware 19901; Emory University, Atlanta, Georgia 30322; and Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

TABLE 2. Distribution of study subject infection status among oyster treatment groups

<table>
<thead>
<tr>
<th>Phase</th>
<th>Treatment conditions</th>
<th>No. of subjects infected/total (%) postchallenge with:</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HPP-treated oysters</td>
<td>Untreated oysters</td>
</tr>
<tr>
<td>1</td>
<td>400 MPa, 25°C, 5 min</td>
<td>3/5 (60)</td>
<td>7/15 (47)</td>
</tr>
<tr>
<td>2</td>
<td>600 MPa, 6°C, 5 min</td>
<td>0/10 (0)</td>
<td>7/15 (47)</td>
</tr>
<tr>
<td>3</td>
<td>400 MPa, 6°C, 5 min</td>
<td>3/14 (21)</td>
<td>7/15 (47)</td>
</tr>
</tbody>
</table>

a The control group represented the combined number of controls over phase 1 through phase 3 (n = 15) because each control received untreated HuNoV-seeded raw oysters with the same amount of HuNoV inoculum.

b Fisher’s exact two-sided test compared each treatment group to all of the controls (i.e., the total number of subjects challenged with non-HPP treated oysters).
The role of vomitus in indirect transmission

Recurring Norovirus Transmission on an Airplane

Craig N. Thornley, Nicola A. Emslie, Tim W. Sprott, Gail E. Greening, and Jackie P. Rapana

1Auckland Regional Public Health Service, Auckland District Health Board, Auckland, New Zealand; 2Air New Zealand Medical Unit, Air New Zealand, Auckland, New Zealand; and 3Environmental Health Food Group, Institute of Environmental Science and Research, Porirua, New Zealand

(See the Editorial Commentary by Lopman, on pages 521-22.)

A norovirus outbreak associated with environmental contamination at a hotel

H. KIMURA1, K. NAGANO2, N. KIMURA3, M. SHIMIZU4, Y. UENO4, K. MORIKANE5 and N. OKABE6

A point-source norovirus outbreak caused by exposure to fomites.

Repp KK, Keene WE.

Washington County Department of Health and Human Services, Hillsboro, OR, USA.
Barriers to Research (Detection)

• Cannot be cultivated outside the human host
• Many viruses, great diversity (no broadly reactive reagents)
• Limited fecal stocks and reagents (VLPs, antibodies)
• Short-lived immunity and currently no vaccine
• Lack of commercially available diagnostics
  – RIDA®-QUICK (R-Biopharm AG) (EIA)
  – CeeramTOOLS® (Ceeram S.A.S.) (RT-qPCR)
  – Others in the pipeline (Qiagen, Cepheid, Shimadzu Corp, Norgen Biotek, Applied Biosystems, others?)

• Limiting factors:
  – No broadly reactive reagents (antibodies)
  – In food/environmental samples: high sample volumes, low contamination levels (10-100 viruses in 25g or 100 ml+)
Simple Concentration
- Homogenization
- Surface Elution

Large volume filtration
- Ultrafiltration
- Glass wool filters

Liquid portion
- Concentration
  - PEG ppt.
  - Acid ppt.
  - Organic Flocculation
  - Cationic particles

Elute from solid

Small volume liquid
- Sample purification
  - Organic solvent extraction
  - Enzymatic digestion

Solid portion

Measures to estimate infectivity

RNA Extraction and Detection
Aptamers vs. Antibodies

**Aptamer**
- Chemical Synthesis
- Modulate binding condition
- Heat stable and recoverable
- Less expensive
- Long shelf-life

**Antibody**
- Animal system
- Cannot modulate binding condition
- Heat sensitive and binding irreversible
- More expensive
- Limited shelf-life
Results: Aptamer Magnetic Capture Method

LoD : \(1 \log_{10} (10)\) Genome Equivalent Copies (GEC)/ml

CAPTURE EFFICIENCY OF SMV BY APTAMERS

Escudero-Abarca et. al. 2014. PLOS One. 9:e106805
Host Susceptibility

- Genetics
  - Histo blood group antigens (HBGAs) and FUT-2 secretor status
- Virus-specific
- Host cell binding receptor or co-receptor

- Host immunity
  - Some, but not broad, cross protection
- Herd immunity
- Quasispecies
- Altered antigenicity

**Table 1 | Virus-like particle binding of synthetic histo-blood group antigens**

<table>
<thead>
<tr>
<th>Genogroup and genotype</th>
<th>Virus-like particle</th>
<th>Year</th>
<th>Synthetic histo-blood group antigen bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.1</td>
<td>Norwalk</td>
<td>1960</td>
<td>A, H1 and H3</td>
</tr>
<tr>
<td></td>
<td>West Chester</td>
<td>2001</td>
<td>A, H1 and H3</td>
</tr>
<tr>
<td>I.2</td>
<td>Southampton</td>
<td>1999</td>
<td>A, H3 and Le^A</td>
</tr>
<tr>
<td>I.3</td>
<td>Desert Shield</td>
<td>1999</td>
<td>Le^A</td>
</tr>
<tr>
<td>I.4</td>
<td>Chiba</td>
<td>2000</td>
<td>A, Le^A and Le^K</td>
</tr>
<tr>
<td>II.1</td>
<td>Hawaii</td>
<td>1971</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Weisbaden</td>
<td>2001</td>
<td>None</td>
</tr>
<tr>
<td>II.2</td>
<td>Snow Mountain</td>
<td>1976</td>
<td>H3</td>
</tr>
<tr>
<td></td>
<td>Buds</td>
<td>2002</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Ine</td>
<td>2002</td>
<td>None</td>
</tr>
<tr>
<td>II.3</td>
<td>Toronto</td>
<td>1999</td>
<td>A and H3</td>
</tr>
<tr>
<td>II.4</td>
<td>GII.4.1907</td>
<td>1987</td>
<td>H3 and Le^Y</td>
</tr>
<tr>
<td></td>
<td>GII.4.1907_D393G</td>
<td>2007^</td>
<td>B and H3</td>
</tr>
<tr>
<td></td>
<td>GII.4.2002</td>
<td>2004</td>
<td>H3 and Le^Y</td>
</tr>
<tr>
<td></td>
<td>GII.4.2004</td>
<td>2004</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>GII.4.2005</td>
<td>2005</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>GII.4.2006</td>
<td>2006</td>
<td>A, B and H3</td>
</tr>
<tr>
<td></td>
<td>M7</td>
<td>1999</td>
<td>None</td>
</tr>
<tr>
<td>V</td>
<td>Mouse norovirus</td>
<td>2004</td>
<td>None</td>
</tr>
</tbody>
</table>


From: Donaldson et al., 2010
• **P2** (hypervariable) domain of capsid sequence likely defines antigenic profile of HuNoV

- Previous research has identified two regions of AA residues associated with receptor binding.
  - Site A (major epitope; 296-298)
  - Site B (minor epitope; 393-395)
Norovirus Recognizes Histo-Blood Group Antigens on Gastrointestinal Cells of Clams, Mussels, and Oysters: A Possible Mechanism of Bioaccumulation

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Development of a Fluorescent In Situ Method for Visualization of Enteric Viruses

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FIG. 2: Visualization of biotinylated virus binding to the surface of onion epidermis. (A) Onion epidermis after exposure to bio-HAV and Q-Dots 655. (B) Onion treated with the Q-Dots only. (C) Onion epidermis after the bio-HAV had been eluted from the surface, with best contrast in image 3. The epidermis is shown in image 1. Image 2 shows the fluorescence from the Q-Dots, and image 3 is an overlay of images 1 and 2.
Summary

• HuNoV: the perfect pathogen?
  – Highly infectious
  – Rapid and efficient spread by a variety of means
  – Environmentally stable and resistant to many sanitizers and processing technologies
  – Constantly evolving
  – Evokes limited immune response
  – Only moderately virulent (rarely lethal)
Summary

• Bottom line:
  – New strains will continue to emerge (they may be associated with more severe disease)
  – Collectively, the unique features of HuNoV allow these viruses to persist in the human population
  – Vaccination options complicated
  – Will likely remain a public health challenge for years to come
The USDA-NIFA Food Virology Collaborative

**Long Term Goal:** To reduce the burden of food borne disease associated with viruses, particularly noroviruses

**Approach:** Multi-disciplinary team working in an integrated manner to develop improved tools, skills, and capacity to understand and control food borne virus risks

**Objectives (Cores):**
- Molecular virology
- Detection
- Epidemiology and Risk Analysis
- Prevention and Control
- Extension and Outreach
- Education and Capacity Building

![Diagram showing the structure of the USDA-NIFA Food Virology Collaborative with the following cores: Detection Core, Epidemiology & Risk Analysis Core, Molecular Virology Core, Control Strategies Core.](image)
Partners in the Food Virology Collaborative
Stakeholder Engagement
Research Activities

• **Molecular Virology**: Develop improved methods to facilitate the study of foodborne viruses

• **Detection**: Develop and validate sensitive, rapid, and practical methods to detect and genotype HuNoV in relevant sample matrices

• **Epidemiology and Risk Analysis**: Collect and analyze population data on the burden of virus-associated foodborne disease, including epidemiological attribution and characterization of risk and costs

• **Prevention and Control**: Improve understanding the occurrence and behavior of HuNoV in the food safety continuum so as to inform development of scientifically justifiable control measures.
Research: Molecular Virology and Detection

- Key limitation to the study of norovirus is that it is uncultivable:
  - can’t grow it in the lab >>> volunteers or stool samples from outbreaks
  - Develop a culture system for this virus in the lab

- Develop sensitive, rapid, and practical methods to detect and genotype human norovirus in relevant sample matrices, namely food products
  - mathematical modelling,
  - develop novel methods of detection, and
  - determining ways to discriminate between infectious and non-infectious virus
Research: Epidemiology/Risk Analyses and Prevention/Control

• develop and apply risk models in order to estimate the economic, endemic, and epidemiological burden of food borne disease caused by human norovirus

• improve the understanding of the occurrence and behavior of human norovirus
  – potential alternative indicator organisms
  – development of novel agents for hand and surface decontamination
  – new mitigation technologies/strategies in foods
Just a few of the ongoing areas of Research:

- Literature database – more than 2,500 articles
- Ongoing work at CDC – repository of reagents to study noroviruses
- Comparison of different norovirus surrogates
- Prevention and Control – Assessment of High Pressure Processing (HPP)
- 25 μl (GI.6 or GII.4 fecal suspension)
- 1 μl (GII.4 VLPs)

Expire on coupons (1x1") for 0 to 240 minutes

Elute by pipetting up and down with PBS-EDTA (20mM)

## Alloys Tested

<table>
<thead>
<tr>
<th>Metal alloy (UNS designation)</th>
<th>Percent copper</th>
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</thead>
<tbody>
<tr>
<td>Copper (C11000)</td>
<td>100</td>
</tr>
<tr>
<td>Bronze (C51000)</td>
<td>95</td>
</tr>
<tr>
<td>Copper-Nickel (C70600)</td>
<td>87</td>
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<tr>
<td>Brass (C26000)</td>
<td>70</td>
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<tr>
<td>Muntz metal (C28000)</td>
<td>60</td>
</tr>
<tr>
<td>Stainless steel (S304000)</td>
<td>0</td>
</tr>
</tbody>
</table>
Rnase-H RT-qPCR (genome integrity)

Transmission electron microscopy (capsid integrity)

HBGA binding assay (capsid function)

SDS-PAGE/Western blot (capsid integrity)
Rnase-H RT-qPCR Results (GI.6)

* P < 0.05

Rnase-H RT-qPCR Results (GII.4)

Log₁₀ reduction

Time (minutes)

Stainless Steel
Brass (70%)
Copper Nickel (87%)
Bronze (95%)
Copper (100%)


* $P < 0.05$
Electron Microscopy Results

- VLPs exposure to SS remain dispersed and stable for 240 minutes exposure
- Exposure to copper results in aggregation (60 minutes) and eventually destruction (240 minutes)
• Rapid loss of HuNoV VP1 capsid protein occurs in samples exposed to copper, but not stainless steel.
Exposing GII.4 VLPs to copper surfaces results in rapid (less than 10 minutes) loss of ability to bind HBGA receptor.

GII.4 VLPs exposed to stainless steel retain HBGA binding ability for several hours (data not shown).

Major Findings

Exposure to copper surfaces results in changes to both capsid and genome integrity, supporting HuNoV destruction.

1. Destroys the HuNoV Capsid
2. Destroys the HuNoV genome
The Big Picture

• HuNoV is destroyed by copper containing surfaces

• Incorporating copper touch surfaces in at risk settings (cruise ships, restaurants, restrooms, elder care facilities) may help reduce HuNoV environmental transmission
  – Use as an additional “tool”, not substitute for sanitation
Extension, Outreach, and Education Activities

- **Extension and Outreach:** Translate and disseminate new knowledge about foodborne viruses into practices that reach target audiences in relevant work environments and across a wide array of stakeholder groups.

- **Capacity Building:** Build scientific and human capacity to support increased and sustained efforts in food virology by fostering information and exchange, expanding professional capacity through formal student education and training initiatives.
Extension Messages for Control of Foodborne Viruses:

• Foodborne outbreaks from microbial pathogens continue to have **significant public health impacts**
  – And significant costs to the industry

• **Viruses** have emerged as the leading cause of foodborne outbreaks of gastroenteritis

• Not all microbial pathogens are created equal…
  – Viruses behave very differently in the environment as compared to bacteria
Extension Messages for Control of Foodborne Viruses:

- Viruses of public health significance come from **HUMAN** fecal contamination
  - Viral pathogens (such as norovirus and Hepatitis A virus) are generally more host specific than parasitic and bacterial pathogens

- Viruses are **shed in high numbers** from infected individuals

- Viruses are **infectious at very low doses**
Extension Messages for Control of Foodborne Viruses:

• Viruses are **stable in the environment and resistant to chemical disinfectants**

• Control strategies can be developed from understanding how viruses are transmitted
  – Through contaminated water
  – Associated with vomiting incidents
  – Particularly on hands of infected workers

• **Prevention is the key for controlling viral pathogens!**
Extension Messages for Control of Foodborne Viruses:

- Promote thorough and frequent handwashing!
- Pay attention personal hygiene and to restrooms!
- Take care to manage vomiting events!
- Do not assume that simple surface disinfection with standard chlorine concentrations will eliminate viruses on surfaces (>1000 ppm)!
Harvesters: keeping your hands clean

Dirty hands can contaminate produce with viruses that cause human illnesses, like hepatitis A and norovirus.

**Prevention** is the best control and good hand hygiene is critical to making berries safer.

- Wash your hands thoroughly with soap and clean water, especially after using the bathroom.
- Do not rely on alcohol-based hand sanitizers; they are not completely effective against foodborne viruses like norovirus and hepatitis A.

Berries are **sticky foods** for viral contamination.

- They are hand picked and these viruses spread easily with hand contact via the fecal oral route (poop to mouth).
- Berries are generally not heated or cooked before being eaten so virus is not destroyed.
- The use of sanitizers, washing, and/or freezing berries is not effective for removing or destroying the virus.

**Foodborne viruses**

Noroviruses are the leading cause of foodborne illness.

- Norovirus (the “stomach flu”) causes nausea, vomiting, and diarrhea. There are over 3 million foodborne cases per year in the U.S. alone. Hepatitis A illness starts with flu-like symptoms and then progresses to jaundice (yellowing of the skin & eyes) and sometimes other complications.
- For both viruses, it is possible to be infected and not show symptoms, the sick person in also infectious for days to weeks before, during, and after illness, so keeping your hands clean is especially important. This is also important if you are taking care of someone who is ill.
- Norovirus infection is miserable but usually lasts a short time. Sometimes it is necessary to see a doctor because of dehydration. Hepatitis A infection is much more severe.

**Outbreak Snapshots**

<table>
<thead>
<tr>
<th>Hepatitis A</th>
<th>Norovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>In 2013, over 100 people in the Western U.S. became ill with hepatitis A infections after eating contaminated frozen berries (pomegranate seeds are likely the vehicle of contamination).</td>
<td>In 2012, over 11,000 children and teens in Germany were sickened by norovirus from contaminated frozen strawberries distributed to schools.</td>
</tr>
<tr>
<td>In early 2013, dozens were sickened in Europe over several months from frozen berries served in smoothies.</td>
<td>In a 2009 norovirus outbreak in Europe caused by raspberries, over half of those affected were children younger than 7 years of age.</td>
</tr>
<tr>
<td>A 2012 outbreak of hepatitis A in Canada was also linked to a frozen mixed berry blend.</td>
<td>In 2005, contaminated raspberries sickened more than 1000 people in Denmark, including people in hospitals and nursing homes.</td>
</tr>
</tbody>
</table>
Hand Hygiene for Farm Management

Dirty hands can contaminate produce with viruses that cause human illnesses, like hepatitis A and norovirus. Farms need adequate toilet facilities and hygiene tools.

Prevention is Key

- **Training**: Educate workers about good hand hygiene practices and proper glove use. Teach control measures, why they are important, and what the consequences are if they are not used. Do not rely on alcohol-based hand sanitizers, they are not completely effective against foodborne viruses.

  Norovirus (the “stomach flu”) causes nausea, vomiting, & diarrhea. Hepatitis A illness starts with flu-like symptoms but progresses to disease of the liver, leading to jaundice (yellowing of the skin & eyes) and sometimes additional complications.

  For both viruses, it is possible to be infected and not show symptoms; the sick person sheds virus for a long time, and these viruses remain stable in the environment.

- **On the Farm**: Provide the facilities. Adequate toilet and handwashing facilities include soap, clean water, and paper towels. Trash bins should not be allowed to overflow (soiled paper or tissue can contaminate shoes).

- **Opportunity**: Create a working environment and schedule that promotes appropriate hand washing practices.

Virus contamination of field worker hands has been linked to illness...

...these viruses can be transferred from hands to produce.

...for proper handwashing, provide soap, clean water, and paper towels.

...they are hand-picked and generally not heated or cooked prior to consumption. Use of emitters, washing, and dressing is not effective for removing or destroying either virus.
Don't Barf off the Boat
Your Vomit Matters

Norovirus: the quick & dirty

Symptoms: nausea, vomiting, diarrhea, stomach pain, sometimes fever and headache

No symptoms does not mean no virus. You can still spread the virus after you recover.

One person's vomit can contain billions of virus particles.

As few as ten particles can make you sick. Your vomit could infect 100s to 1000s of people.

Healthy people usually recover from norovirus in a few days. For children and the elderly the illness can be severe.

But you have to puke somewhere.

Do it in...
a flushable toilet (lid down when you flush) or a container you can seal & throw out/disinfect with liquid bleach.

Clean it up...
with disposable paper towels and seal them in a plastic bag to throw out

... disinfect the affected area and all surrounding areas up to 6 feet beyond

use chlorine bleach concentrated at 1/3 cups liquid bleach/1 gallon of water

let it sit for at least 5 minutes
repeat if possible, then clean as usual.

Always wash your hands with soap and water, especially after using the bathroom or cleaning up vomit. Wash affected clothing and linens immediately.
Don’t Poo in the Blue...

Human sewage in the ocean can cause human illnesses.

What happens?

Norovirus (the “stomach flu”) causes nausea, vomiting, diarrhea, stomach pain, and sometimes fever. There are millions of cases each year in the U.S. alone, resulting in 1,000s of hospitalizations and 100s of deaths. It spreads through the fecal-oral route (poop to mouth), by way of food, water, objects, surfaces, and other people.

Norovirus particles are small and can survive in the environment for a long period of time without losing the ability to infect people. Oysters and clams filter water, concentrating Norovirus in their bodies. The virus can remain in the environment for a long period of time. The oysters and clams harvested and served, usually raw or lightly steamed. This light cooking does not inactivate norovirus, and people can become ill. They may then spread the virus to others.

Your poop matters.

Just one person’s poop is enough to cause an outbreak.

- 1 gram of poop, about the weight of a fish hook, can contain millions of virus particles.
- It only takes 10-100 viruses to get sick.
- The waste from one person can contaminate an area about the size of 25 football fields.

Boaters once contaminated a U.S. Gulf Coast waterway by dumping human waste overboard. Oysters harvested from that area caused a norovirus outbreak that sickened 200 people across 6 states. It only took this one incident to cause the outbreak.

Reduce the Risk

1) Know the symptoms of norovirus and stay off the boat while ill.
2) Poop with care. Use a toilet or container for poop and dispose of the contents at marina stations if possible.
3) Disinfect all items that have contact with poop. Use 1.5 cups of liquid chlorine bleach per gallon of water and let it sit for at least 5 minutes. Repeat disinfection then clean as normal.
4) Wash hands with soap and water. Do this often, especially after using the bathroom. Do not rely on hand sanitizers alone, they are not completely effective against norovirus.
Prevent Poo in the Blue
A Bulletin for Marinas

What happens...

Boat heads are emptied in waterways or people poop directly in the water. This waste may contain norovirus particles. Infected people can shed the virus even when they are not showing symptoms.

Oysters and clams filter viruses from the contaminated water, concentrating them in their bodies. Noroviruses can remain in the environment for a long period of time without losing the ability to infect people.

The oysters and clams are harvested and served, usually raw or lightly steamed. This light cooking does not inactivate norovirus, and people can become ill. They may then spread the virus to others. People usually recover without problems, but dehydration is a concern, and may rarely result in hospitalization.

Poop matters.

Just one person’s poop is enough to cause an outbreak.
- 1 gram of poop, about the weight of a fish hook, can contain millions of virus particles.
- It only takes 10-100 viruses to get sick.
- The waste from one person can contaminate an area about the size of 25 football fields.

Human sewage in the ocean & waterways can cause human illnesses such as norovirus (the “stomach flu”), which causes nausea, vomiting, diarrhea, stomach pain, and sometimes fever. There are millions of cases each year in the U.S. alone, resulting in 1,000s of hospitalizations and 100s of deaths. It spreads through the fecal-oral route (poo to mouth), by way of food, water, objects, surfaces, and other people.

Reduce the Risk

1) Raise awareness. Post information about these risks where boaters can see it.
2) Provide functional facilities for boat waste disposal.
3) Post information about proper handwashing practices.
   - Hands should be washed with soap and water, and dried with paper towels.
   - Hands should be washed often, especially after using the bathroom.
   - Do not rely on hand sanitizers alone, they are not completely effective against norovirus. Provide handwashing facilities.

Boaters once contaminated a U.S. Gulf Coast waterway by dumping human waste overboard. Oysters harvested from that area caused a norovirus outbreak that sickened 200 people across 6 states. It only took this one incident to cause the outbreak.
Extension/Outreach Partnership with the Interstate Shellfish Sanitation Conference

- NoroCORE convened an expert advisory panel in February, 2014
  - Representation on ISSC, FDA, USDA, Sea Grant, and States

- Consensus that the focus of an outreach and educational program should be **PREVENTION** of focal contamination events in close proximity to the shellfish growing waters

- Collaborative partnership to establish a multi-tiered approach for disseminating information about viral- and other microbial-related risks to a wide range of shellfish stakeholder groups, including:
  1. Commercial Fishermen
  2. Recreational Boaters
Extension/Outreach Partnership with the ISSC

1. obtain and provide the necessary computer software to “harvesting states”, enabling them to modify existing ISSC Harvester and Dealer Training Program templates and design training materials to meet the NSSP requirements for shellfish harvester and dealer training;

2. provide technical support and assistance to “harvesting states” as necessary, for them to design and update the ISSC Harvester and Dealer Training Program templates with their state-specific information;

3. update the existing ISSC educational DVD that focuses on use of pump stations to reduce illicit overboard waste dumping, by adding virus-related content for outreach to molluscan shellfish stakeholder groups;
Extension/Outreach Partnership with the ISSC

4. co-sponsor a national conference hosted by the ISSC to share information about viral illnesses associated with molluscan shellfish and novel alternate indicators that may be used for managing shellfish growing and harvest waters; and

5. develop and conduct a survey to “harvesting states” to assess how best to disseminate educational information to recreational boaters about microbial contamination of molluscan shellfish growing and harvest waters.
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  • Senior researchers: Dr. Helen Rawsthorne, Dr. Rebecca Goulter, Dr. Blanca Escudero-Abarca
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  • NoroCORE staff: Malakai Erskine, Dr. Christina Moore

• Academic Members of the USDA-NIFA Food Virology Collaborative

• Industry and Industrial Stakeholders

• Funding Agencies
  • ILSI-NA
  • USDA Food Safety Safety Research Programs
  • Merieux Alliance Foundation
Helpful links

- NoroCORE  
  [http://norocore.ncsu.edu/](http://norocore.ncsu.edu/)
- CDC norovirus  
  [http://www.cdc.gov/norovirus/](http://www.cdc.gov/norovirus/)
- CaliciNet  
- CDC Vital Signs  
- Clean Hands Save Lives  
  [http://www.cdc.gov/handwashing/](http://www.cdc.gov/handwashing/)
- Vessel Sanitation Program  