Molecular Epidemiology of Noroviruses

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Infectious Diseases

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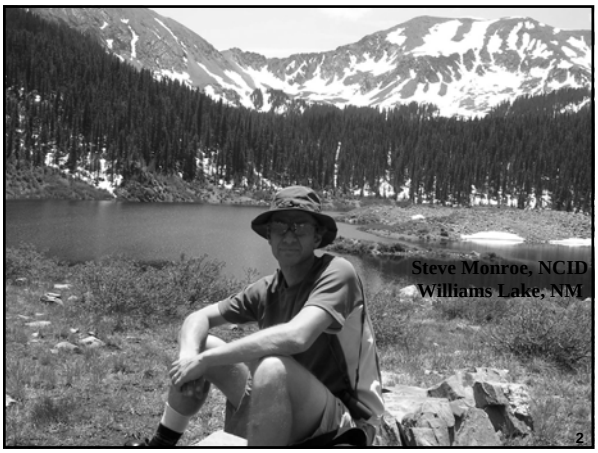

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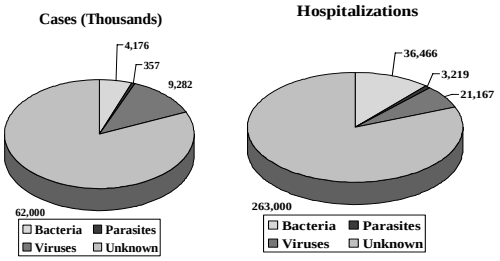
Role of Viruses in Sporadic Foodborne Illness



Source: Mead, et al Emerging Infectious Diseases 5:607-625:1999

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Sporadic Norovirus Gastroenteritis: Emergency Department Patients (1999-2001)

- 3 FoodNet sites (CT, NY, OR)
- 364 subjects enrolled
- 152 subjects with stool sample tested for all pathogens
- Norovirus most common pathogen detected
  - 20% positive by RT-PCR
  - Additional 12% positive when serologic diagnosis is included

Pathogen	No. positive (%)
Viruses	49 (32%)
Norovirus	**30 (20%)**
Rotavirus	18 (12%)
Any bacteria	21 (14%)
Any parasite	3 (2%)
Any pathogen	73/152 (48%)
No pathogen	79/152 (52%)

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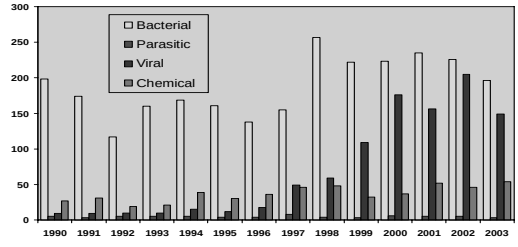
Foodborne Outbreak Surveillance



Source: Foodborne Outbreak Reporting System
http://www.cdc.gov/foodborneoutbreaks/

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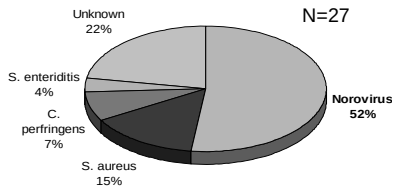
Enhanced Foodborne Outbreak Surveillance: CA, MD, TN; 2001-2002



- Active Outbreak Detection
- Aggressive sample collection (In-home kits)
- Comprehensive pathogen testing

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Viruses: The "Other" Enteric Pathogens

- No replication outside of human host
  - No animal reservoir
  - Must detect directly in clinical or env samples
  - Detection = adulteration
- Vigorous replication inside of human host
  - Low infectious dose
  - High yield (excrete 10^{10} infectious doses)
- Small on the inside, hard on the outside
  - Difficult to disinfect

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Viruses of Gastroenteritis

- Rotavirus
  - Groups A, B, and C
- Adenovirus
  - Primarily Group F (types 40 & 41)
- Astrovirus
- Calicivirus
  - Norovirus (NoV)
    aka: Norwalk-like viruses (NLV), small round structured viruses (SRSV)
  - Sapovirus (SaV)
    aka: Sapporo-like viruses (SLV), "classic human caliciviruses"
- Poorly Characterized Viruses
  - Coronavirus, Picobirnavirus

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Taxonomy Update: Family *Caliciviridae*

- Genus *Norovirus* (NoV)
  - Type species *Norwalk virus*
  - Aliases: "Norwalk-like Virus" (NLV), Small Round Structured Viruses (SRSV)
- Genus *Sapovirus* (SaV)
  - Type species *Sapporo virus*
  - Aliases: "Sapporo-like Virus" (SLV), "Classic Human Caliciviruses"
- Genus *Vesivirus*
  - Type species: feline calicivirus
- Genus *Lagovirus*

Source: ICTV, Seventh Report; Minutes of XII ICV

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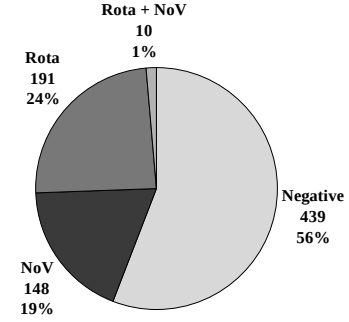
Enteric Viruses in Finnish Children Placebo Group (N=788)



Source: Pang, et al, Pediatr Infect Dis J, 18:420-426, 1999

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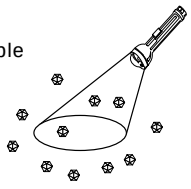
Norovirus Diagnostic Gap: Why is such a common illness so rarely diagnosed?

- Sporadic cases aren't reportable
- Outbreaks rarely investigated
- Samples aren't collected
- Routine testing isn't available (no clinical or commercial labs)
  - No cell culture system or small animal model



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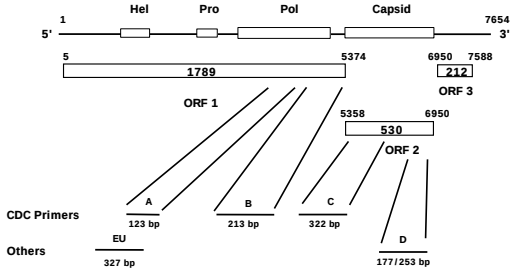
Norovirus Detection by RT-PCR



Source: GenBank M87661

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Why Sequencing?

- Confirmation of standard RT-PCR results
- Limitations to RT-PCR
  - Does not identify genogroup (Region B) or cluster information
- Clarification of the epidemiology of transmission in outbreaks

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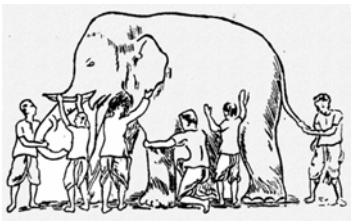
A General Caution!



Molecular sub-typing is a bit like the parable of the blind men and the elephant – you can get an entirely different picture of what you're dealing with depending on which part of the beast you're examining!

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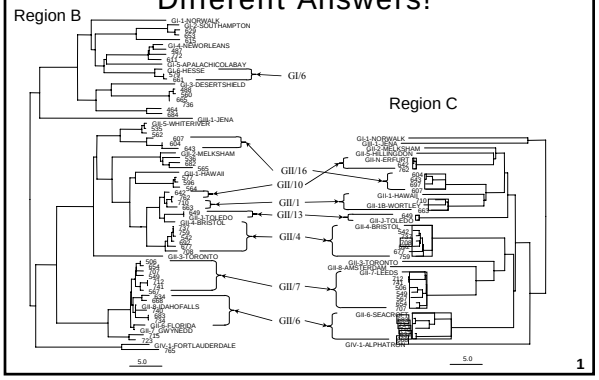
Different PCR Targets, Different Answers!



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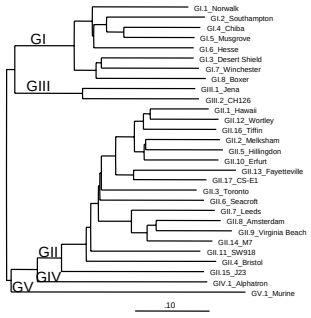
29 Clusters of Noroviruses (Analysis of Capsid Proteins)



Clusters differ by ≥ 20% amino acid pairwise distance

Genogroups differ by 44-55% amino acid pairwise distance

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Characteristics of NoV Epidemiology

- Transmitted through multiple routes
  - Foodborne, Waterborne, Person-to-Person, Mixed
- Outbreaks difficult to control due to:
  - Very common
  - Very low infectious dose
  - Viral shedding can occur for a prolonged period of time and in the absence of clinical illness
  - Environmental persistence
  - Resistance to common disinfectants
  - Substantial strain diversity

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Recent Examples of Noroviruses in Foods

- On the "Farm"
  - Oysters contaminated with NoV (FL, LA & CA)
  - Raspberries contaminated with NoV (Canada, Europe)
- At your Table
  - **Deli meats at university cafeteria (TX)**
  - Pastries in an Army Mess Hall (TX)
  - Potato salad at catered luncheon (AK)
  - "Banquet-in-a-Box" sent to automobile dealers
  - **Frosting on cakes at grocery store (GA**)
  - **Catered luncheon (Netherlands)**
  - **Wedding cakes (MA)**

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Molecular Epidemiology of Foodborne Norovirus Outbreaks (1)

- University Dining Hall (TX, March 1998)
  - Acute AGE associated with lunch or dinner from dining hall deli-Bar
  - 23 students hospitalized
  - 9 / 18 stools positive for NoV
  - Foodhandler's child symptomatic, RT-PCR positive
  - Ham sample from deli bar RT-PCR **positive**, sequence matched that from patient stools
- Source:
  - Daniels, et al. JID, 181:1467-1470, 2000
  - Schwab, et al. App. & Envir. Microbiol. 66(1):213-218, 2000

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Let Them Eat Cake !

WEDNESDAY February 16, 2000

Gwinnett Daily Post 50¢

Cake culprit: Norwalk Virus

Commissioner of Agriculture: Virus a 'highly contagious' food-borne pathogen

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Molecular Epidemiology of Foodborne Norovirus Outbreaks (5)

- Grocery Store Cakes (GA, Feb 2000)
  - AGE associated with eating specialty cakes prepared in a grocery store bakery
  - 153 of 195 attendees from 38 events ill
  - Decorated cakes implicated vehicles (OR=22.2)
  - One foodworker admitted to being symptomatic
  - 15 of 15 stool samples positive for NoV by RT-PCR
  - Sequences of 3 PCR products identical
  - Food samples negative by RT-PCR

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Molecular Epidemiology of Foodborne Norovirus Outbreaks (6)

- Catered Luncheon - Netherlands, 2001
  - 250 / 800 sick
  - Illness associated with eating lunch rolls (OR=1.7)
  - Baker vomited in sink before hand-slicing rolls
  - 24 of 27 stool samples positive by RT-PCR
  - Identical sequence in baker, family and cases

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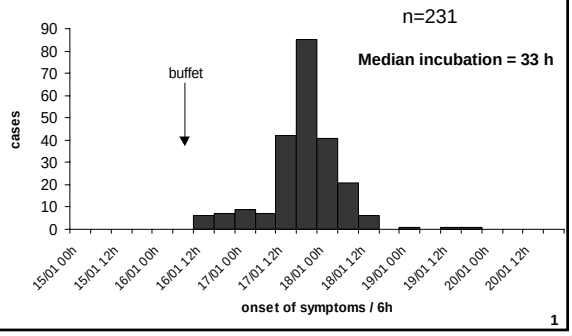
Cases of AGE by 6-hourly Time Period, the Netherlands, 2001



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Molecular Epidemiology of Foodborne Norovirus Outbreaks (7)

- Wedding Cakes (MA, Apr 2002)
  - AGE associated with eating wedding cakes prepared by a single bakery
  - 332 of 850 attendees from 12 events ill
  - (Projected total ~2,700 cases from 46 events)
  - Wedding cakes implicated vehicles (OR=4.5)
  - Two foodworkers admitted to being symptomatic
  - Sequences of 4 PCR products identical

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Multi-State Outbreak Potential

- NoV is so common that we can not identify multi-state outbreaks without molecular epidemiology
- Point Source
  - Individuals infected with NoV in one location and then travel to another area (e.g. conference or cruise ship)
- Wide-spread common exposure
  - Multiple individuals infected with NoV over large geographic regions (e.g. contaminated produce or oysters)

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Cruise Ship Outbreaks

- Multiple points of exposure and transmission methods
  - Brought on by passengers and crew
  - Contaminated food or water
- Multiple co-circulating strains within the population ("mixing bowl")

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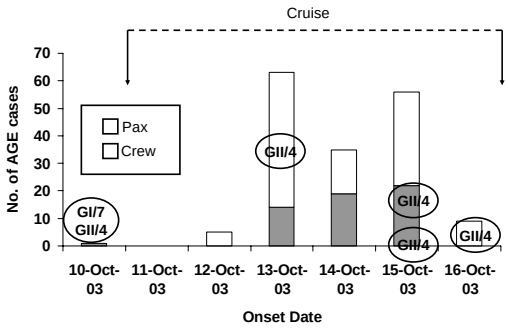
AGE in Passengers and Crew during one 5-day cruise on ship A



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Cruise Ship A

- Five-day Caribbean cruise
- Reached outbreak levels: 5.8% pax and 7.8% crew
- Overall symptom profile:
  - 83% vomiting
  - 76% diarrhea
  - 7% fever
- Multiple strains detected
- On subsequent 4-day cruise: 0.8% pax and 3.7% crew
  - Identical GII/4 sequence from previous cruise

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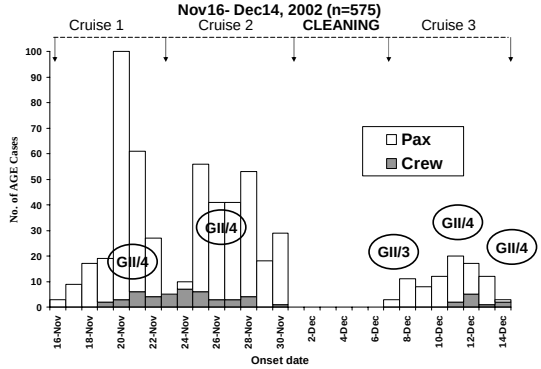
AGE Among Passengers and Crew during consecutive cruises on ship B, Nov16- Dec14, 2002 (n=575)



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Cruise Ship B

- Seven-day Caribbean cruises
  - First cruise: 4% pax
  - Subsequent cruise: 8% pax and 2.3% crew
- One strain detected in pax and crew
  - One case from the first cruise was the suspected index case in a nursing home (126 residents and 49 staff ill)
- Ship was docked for one week for extensive cleaning
- On third cruise, three strains of NoV detected
  - Including the GII/4 sequence from the previous two cruises

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Common Source Outbreak

- Several sushi chefs visited a hotel in Nevada for food preparation lessons in March 2004
- During this time, an acute gastroenteritis outbreak was occurring among hotel patrons
- Three chefs became ill during their visit
- Upon their return to Hawaii, a waitress and patrons of the restaurant became ill after eating the sushi prepared by one of the chefs who visited the Nevada hotel

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Common Source Outbreak

- Stool was collected from the ill restaurant patrons in Hawaii, sushi chef, and Nevada hotel patrons
- Identical nucleotide sequence in the sushi chef, Hawaiian restaurant patrons, and several persons in the Nevada hotel outbreak

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Imported Raspberries

- Canada, 1997*
  - Several clusters of disease in Quebec City in July-August 1997
  - GI illness linked to consumption of raspberry mousse with raspberry sauce (2 cohorts)
  - More than 200 people ill after eating a raspberry dessert
  - Common ingredient: raspberries imported from Bosnia

*Gaulin et al, Can J Public Health 1999 Jan-Feb; 90(1):37-40

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Imported Raspberries

- Canada, 1997
  - Identical NoV strain sequences found in stools of cases and raspberry sauce
  - Similar outbreak traced to frozen raspberries in Finland (1998) and France (2005)

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Outbreak of norovirus infection associated with the consumption of frozen raspberries, France, March 2005

Benoît Cotterelle[1], Corinne Drougard[1], Jacqueline Rolland[2] Monique Becamel[2], Marc Boudon[3], Stéphane Pinede[3], Ousmane Traoré[4], Katia Balay[5], Pierre Pothier[5], and Emmanuelle Espié[6] (e.espie@invs.sante.fr)

[1]Cellule Interrégionale d'Epidémiologie d'Intervention d'Auvergne, Clermont-Ferrand, France
[2]Direction départementale des actions sanitaires et sociales Haute Loire, Clermont-Ferrand, France
[3]Direction départementale des services vétérinaires Haute Loire, Clermont-Ferrand, France
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[5]Centre National de Référence des virus entériques, Centre Hospitalo-Universitaire, Dijon, France
[6]Institut de Veille Sanitaire, St Maurice, France

An outbreak of gastrointestinal illness in students who ate in a secondary school canteen in the administrative département of Haute-Loire, central France was reported to the local health authorities on 23 March 2005. The school has 30 teachers and 334 students, of whom 298 ate lunch at the school canteen. On 24 March, using a standardised questionnaire, students and teachers were asked about recent gastrointestinal illness, and food and drink consumption. A case was defined as a student or teacher at the school who had diarrhoea or vomiting accompanied by at least one other symptom (nausea, abdominal pain, vomiting or diarrhoea) since 21 March 2005. Students who reported recent gastrointestinal illness were asked to submit stool specimens for laboratory testing. A review of food handling procedures was carried out in the school restaurant, and samples of the meals served on 21 and 22 March were tested for common foodborne pathogens.

Of the 270 students and teachers interviewed, 75 (28%) met the case definition. Of the 75 cases, 69 (92%) reported abdominal pain, 59 (79%) vomiting, 53 (71%) nausea, 38 (51%) diarrhoea and 15 (20%) fever. None of the ill students or teachers was admitted to hospital, and all recovered. Duration of illness ranged from less than 1 day to 2 days. Incubation periods, calculated as the time interval between lunch on the 21 March and onset of symptoms, ranged from 12 hours to 36 hours, with a mean of 26 hours and a median of 27 hours.

Consumption of raspberries with fromage blanc (a fresh cheese similar to quark or cottage cheese), a dessert served at lunch on 21 March, was significantly associated with illness (relative risk (RR) 3.3; 95% confidence interval (CI) 1.5–7.5), and was reported by 69 of 74 cases (93%) for whom questionnaires were answered completely. The consumption of fromage blanc alone was not associated with illness (RR 1.8; 95% CI 0.4–9.0). Five of the 6 stool specimens submitted by the students tested positive for norovirus genogroup 1 genotype 5 (Musgrove virus, a

Source: http://www.eurosurveillance.org/ew/2005/050428.asp

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Linking Outbreaks

- Norovirus outbreaks that are clustered in temporally and geographically

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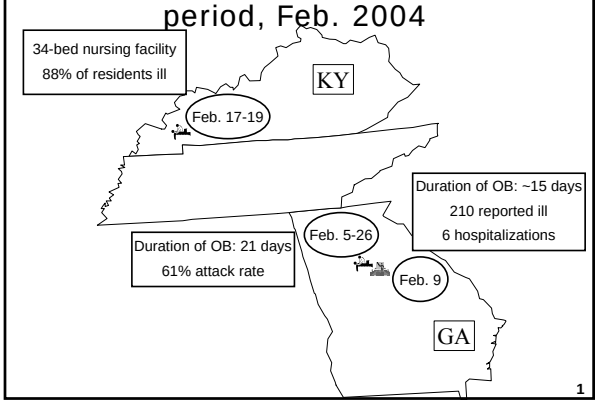
Cluster of GII/4 NoV in a 2-week period, Feb. 2004



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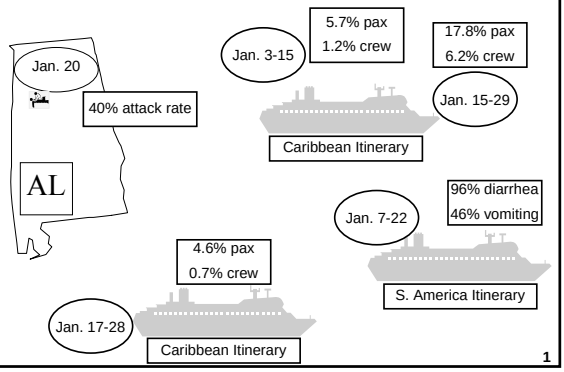
Cluster of GII/4 NoV in a 3-week period, Jan. 2005



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Interpretation

- Questionable genuine clusters
  - No later outbreaks were found where that particular norovirus strain was implicated
- Difficult to interpret with limited information, but does offer tantalizing information about the potential for linking outbreaks to a common source

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Strain Emergence

- In late 2002, a sharp increase in the number of outbreaks was observed on both land and on cruise ships
- One strain found to predominate during this season
- Provisionally named the “Farmington Hills” strain

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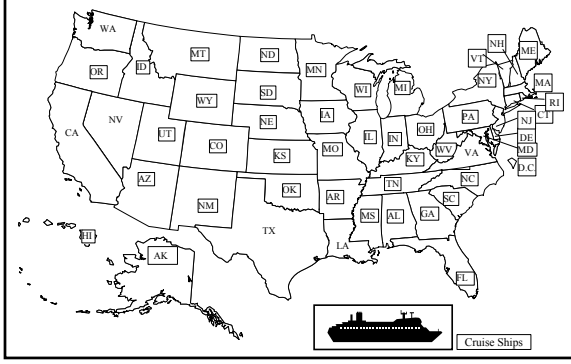
Spread of the Farmington Hill Strain: April 2002 - February 2004



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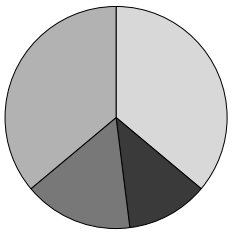
Farmington Hills strain by setting, 2002-2004 (N=25 outbreaks)



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Symptom Frequency

- Compared frequency of diarrhea and vomiting of OBs due to the Farmington Hills strain (n=28) to outbreaks due to other norovirus strains (n=89)
- Individuals in the 28 outbreaks caused by the Farmington Hills strain were slightly more likely to be ill with diarrhea (87% vs. 80%, p=.03) but no difference in frequency of vomiting (73% vs. 76%, ns)

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Limitations

- Only analyzing a small portion of the NoV genome
  - Region B: difficult to differentiate strains
  - Region D: only a portion of the capsid gene
- Predominant emerging strains limits the ability to link outbreaks
- Difficulties in obtaining sequencing information for foods and water

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Conclusions

- Norovirus is the leading cause of sporadic cases and outbreaks of AGE in adults
- Transmitted by multiple routes
- Multiple strains co-circulate; genetic characterization of strains is essential for distinguishing and linking cases
- NoV is so common that we can not easily identify multi-state outbreaks without molecular epidemiology
- Future goals: Rapid Dx assays (e.g. real-time RT-PCR; increased surveillance; increased strain characterization

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Acknowledgements

- Viral Gastroenteritis Lab
  - Susan Adams
  - Suzanne Beard
  - Leslie Hadley
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  - Angie Trujillo
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  - Joe Bresee
  - Lenee Browne
  - Marc-Alain Widdowson
- Many, Many State & Local Lab & Epi

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