



# Molecular Epidemiology of Noroviruses

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# Outline

- Background
- Diagnostics
- Nucleotide Sequencing Uses
- Multi-state Clusters
- Conclusions

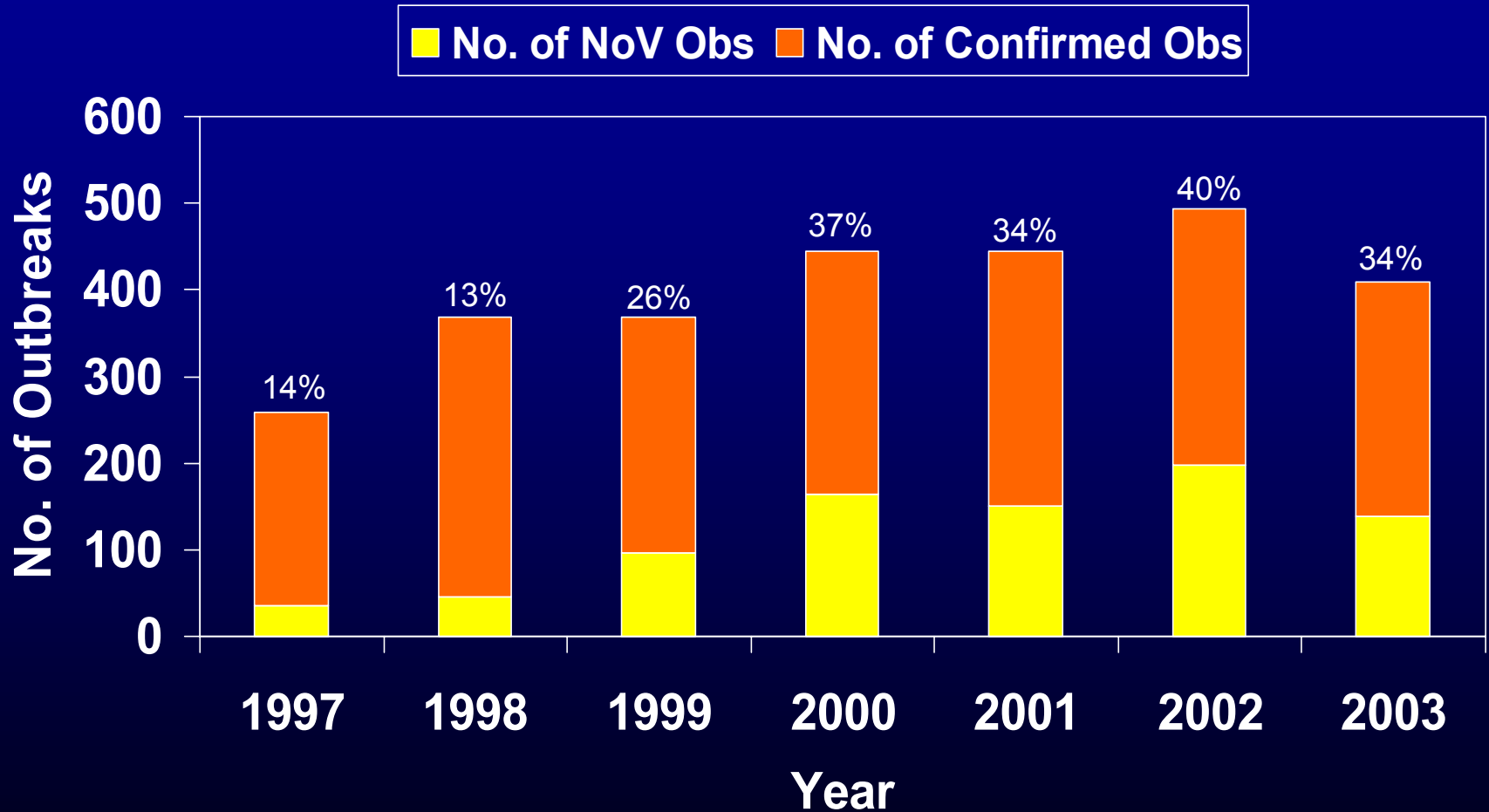
# NoV Etiology

- Avg. incubation period: 12-48 hours (med: 33 hrs)
- Avg. duration: 12-60 hours
- Characteristics: acute onset nausea, vomiting, watery non-bloody diarrhea, abdominal cramps, low-grade fever
- All ages affected
- Dehydration is the most common complication, especially in the elderly and very young

# Disease Burden

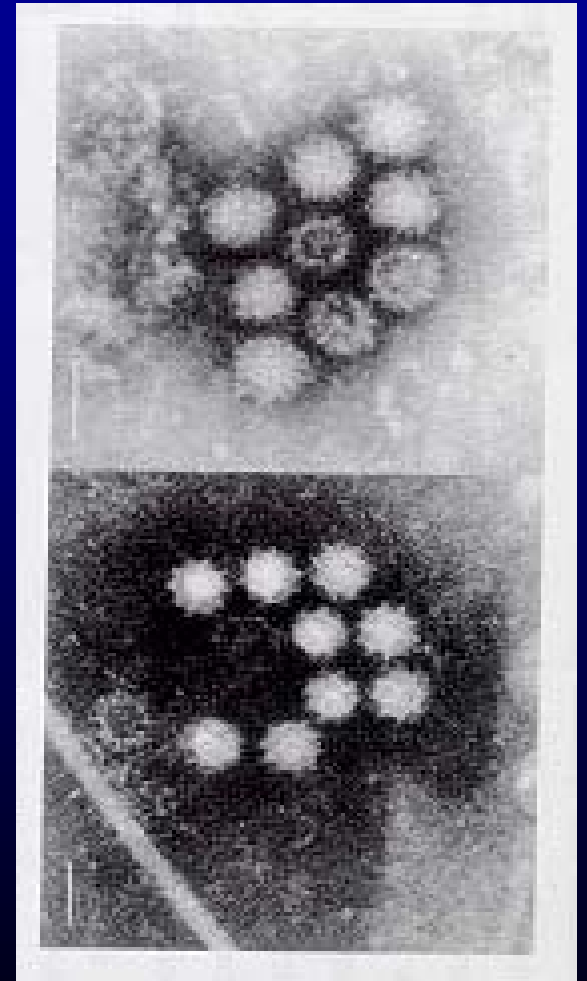
- Major cause of acute gastroenteritis (AGE) in the United States
  - ◆ 23 million cases of illness/year in the U.S. (Mead et al, 1999)
  - ◆ 9.2 million cases of foodborne illness/year in the U.S. (Mead et al, 1999)
  - ◆ 50% of foodborne OBs attributed (Widdowson et al, 2005)

# Norovirus (NoV) confirmed foodborne outbreaks reported to CDC, United States, 1997-2003

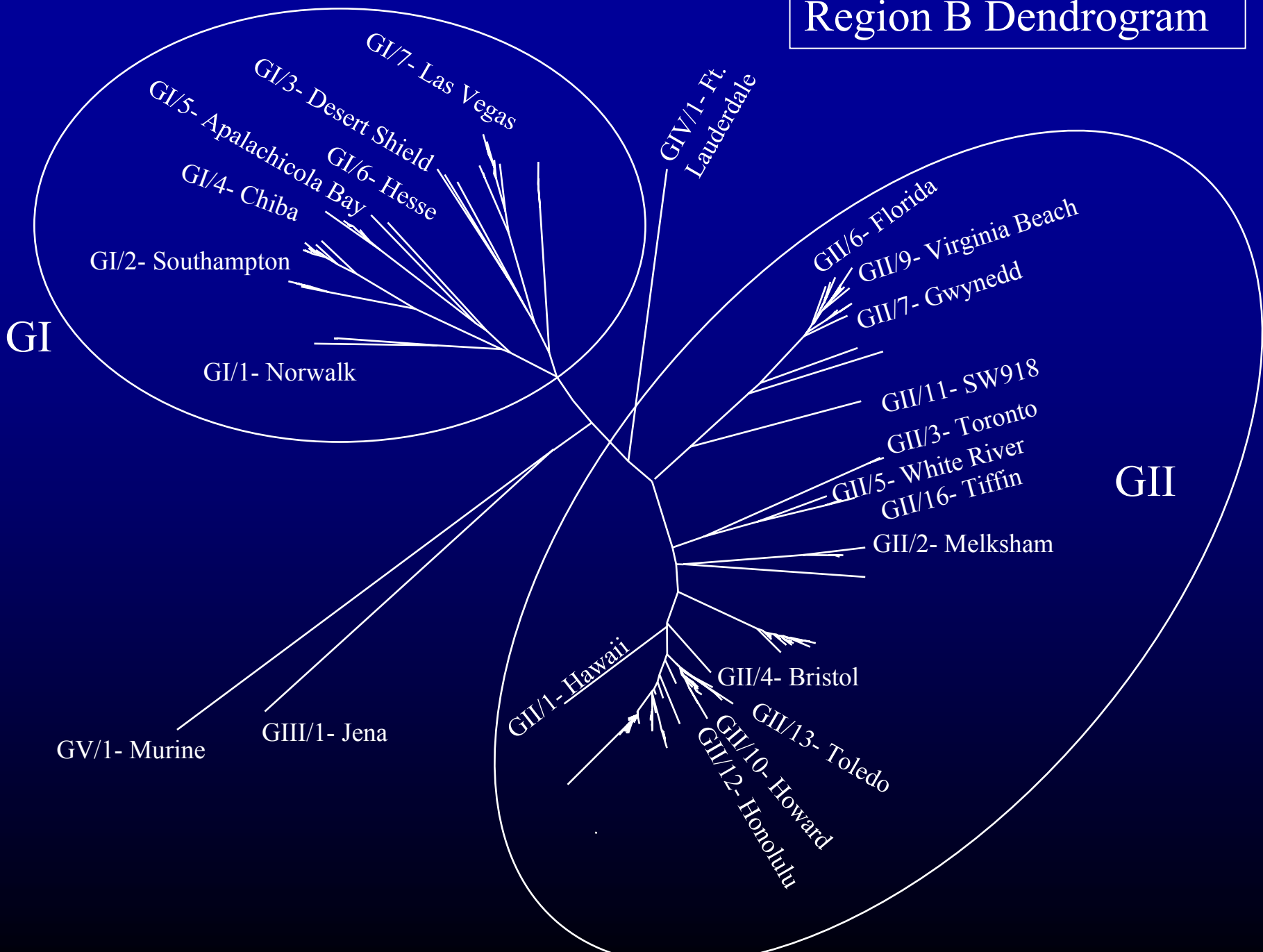


# NoV Strain Diversity

- Genetically and antigenically diverse strains
- Divided into 5 distinct genogroups

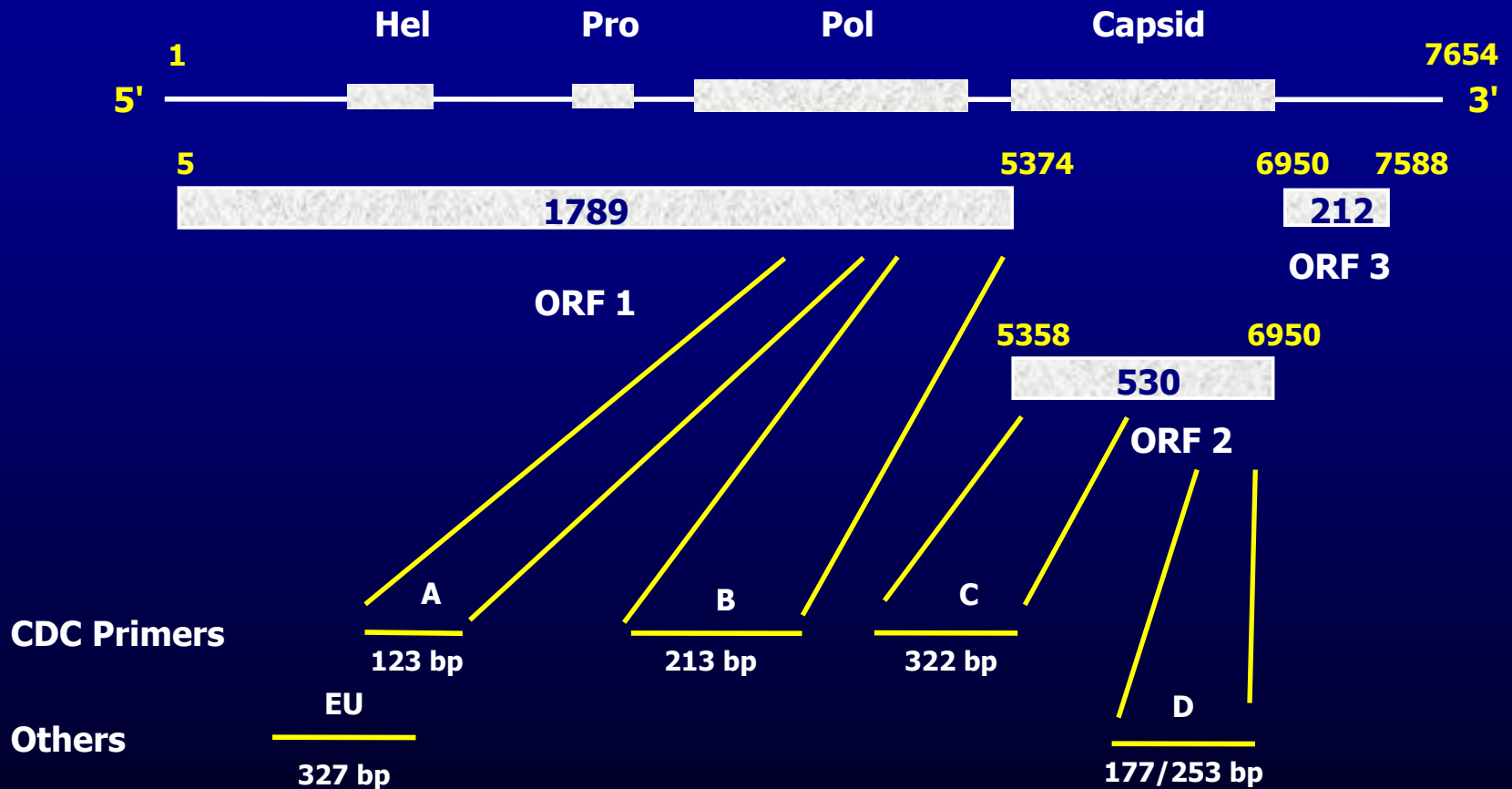


# Region B Dendrogram



# Diagnostics

# Norovirus Detection by RT-PCR



Source: GenBank M87661

# Why Sequencing?

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

# 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

# Nucleotide Sequencing Uses

# 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)

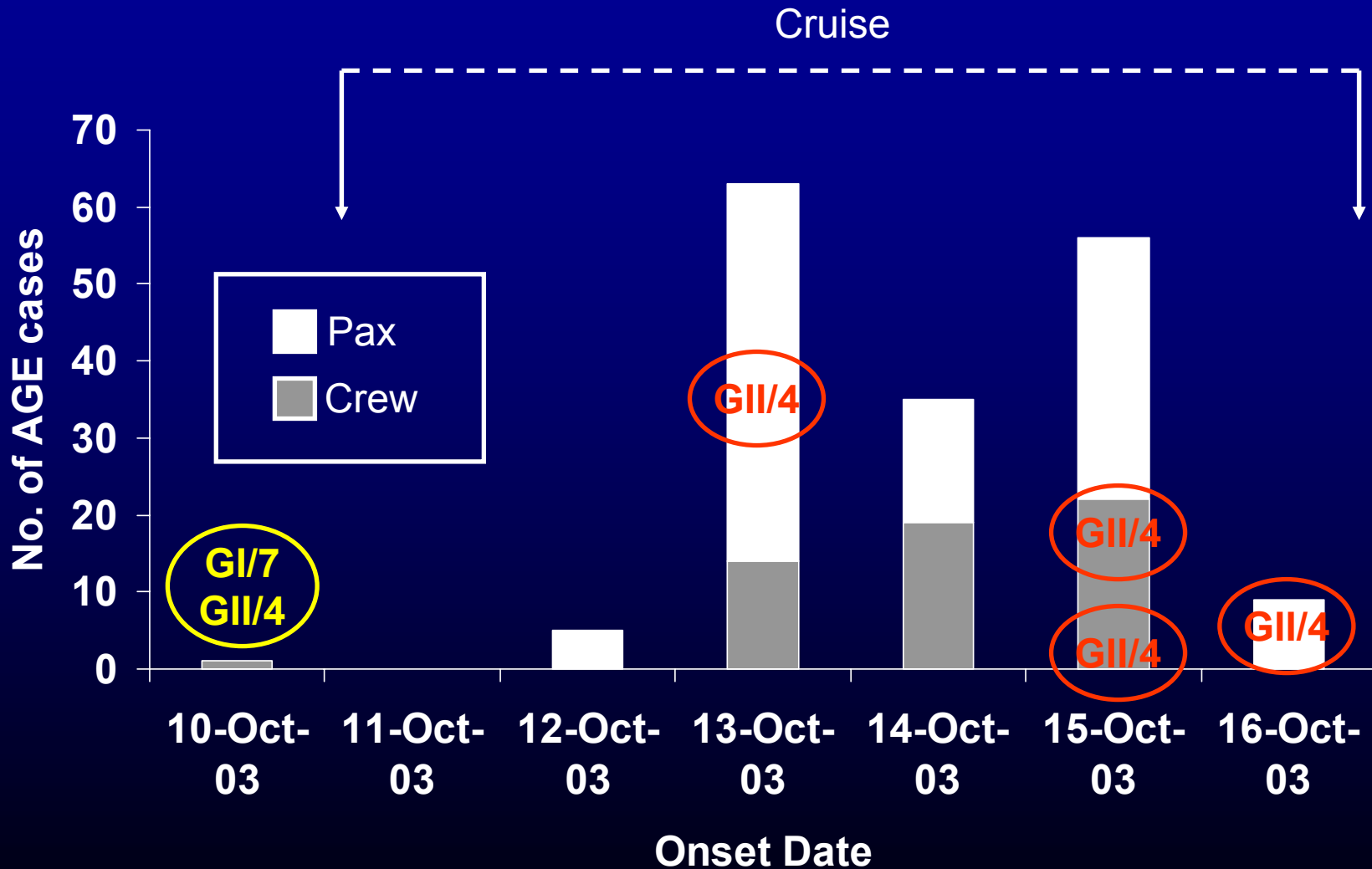
# Sequencing and Epidemiology

- Nucleotide sequencing analysis
  - ◆ Distinguish cases within an outbreak

# 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”)

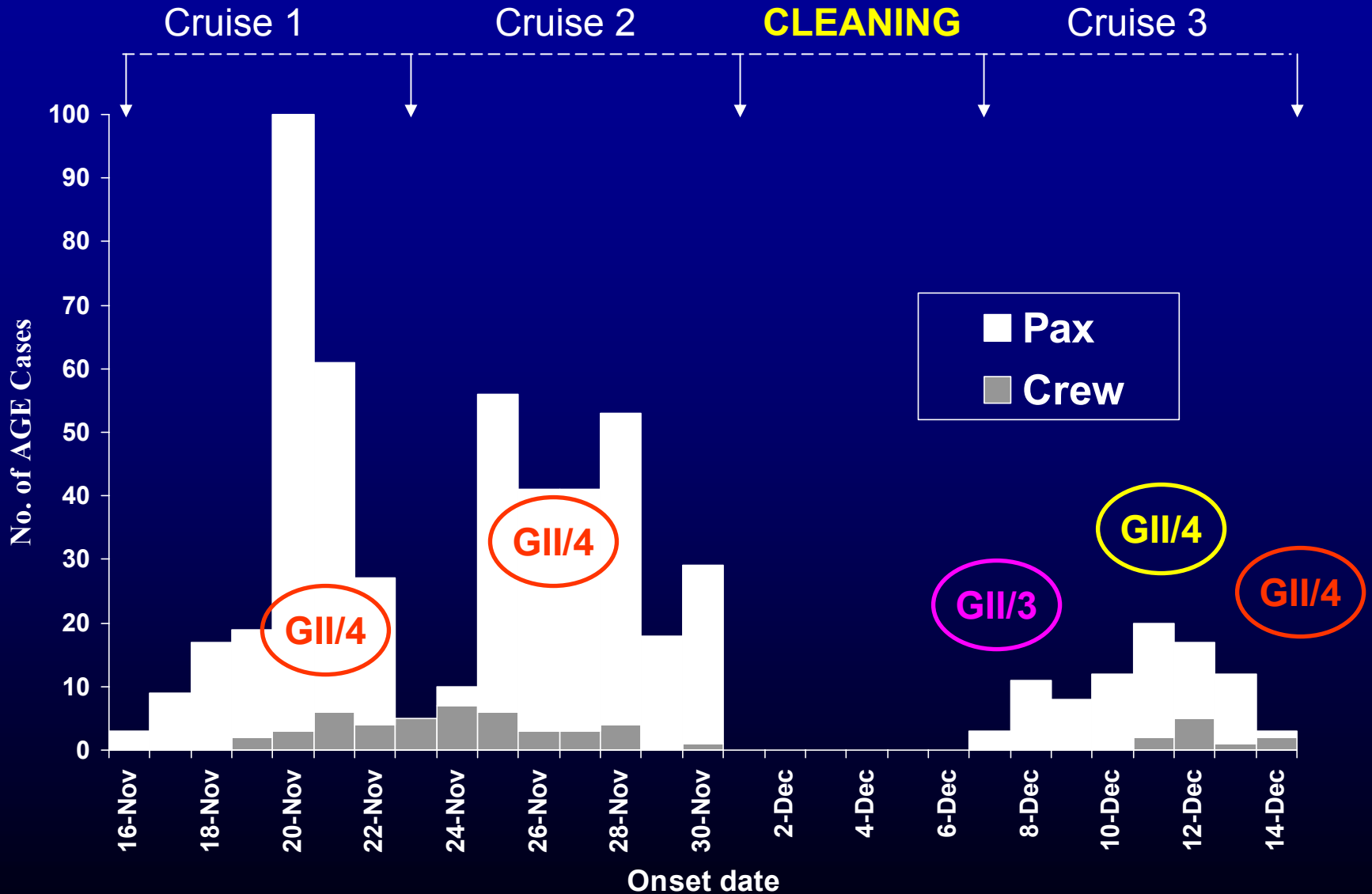
# Numbers of passengers and crew reporting to the ship infirmary with symptoms of AGE during one 5-day cruise on ship A



# 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

# Numbers of passengers and crew reporting AGE during four consecutive cruises on ship B, Nov16- Dec14, 2002 (n=575)



# 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

# Sequencing and Epidemiology

- Nucleotide sequencing analysis
  - ◆ Links to multi-state outbreaks

# 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

# 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

# Sequencing and Epidemiology

- Nucleotide sequencing analysis
  - ◆ Contaminated vehicles

# Contaminated Produce

## ■ 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

# Contaminated Produce

## ■ 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)

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

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

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<sup>6</sup>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 56 hours, with a mean of 36 hours and a median of 37 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 I genotype 5 (Musgrove virus, a

# Multi-State Clusters

# Linking Outbreaks

- Norovirus outbreaks that are clustered in temporally and geographically

# Cluster of GII/4 NoV using Region C primers in a 2-week period, Feb. 2004

34-bed nursing facility  
88% of residents ill

Feb. 17-19

KY

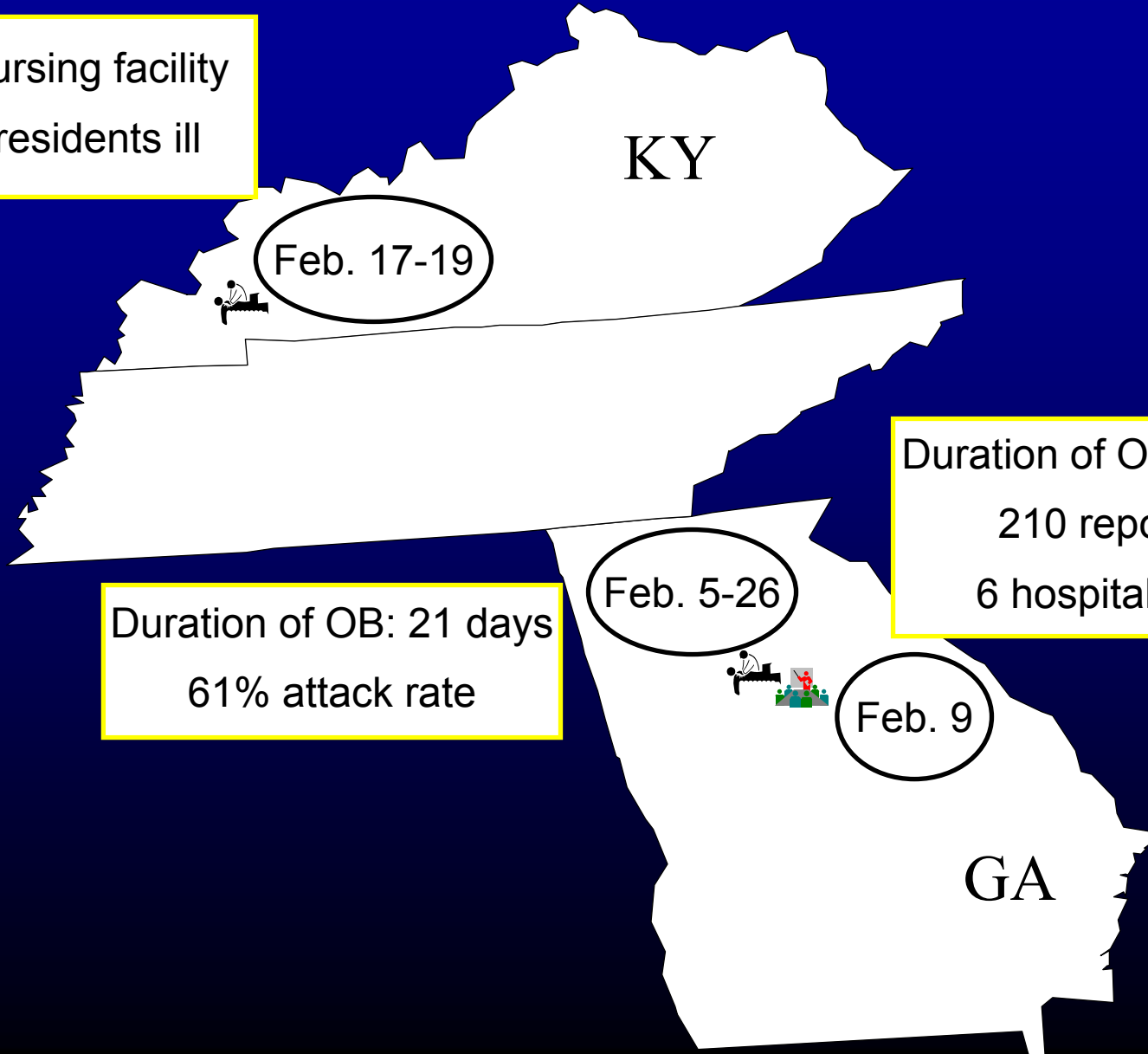
Duration of OB: ~15 days  
210 reported ill  
6 hospitalizations

Duration of OB: 21 days  
61% attack rate

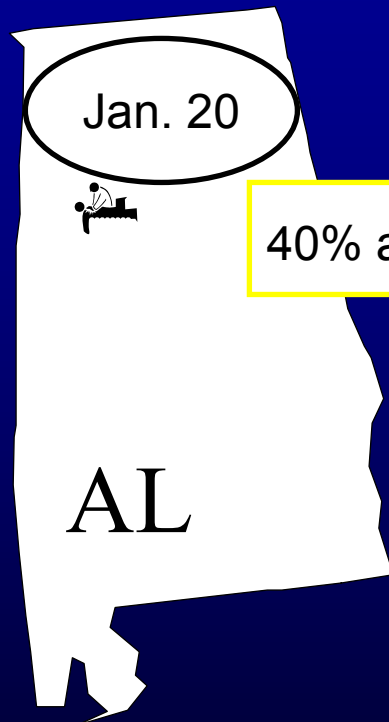
Feb. 5-26

Feb. 9

GA



# Cluster of GII/4 NoV using Region D primers in a 3-week period, Jan. 2005



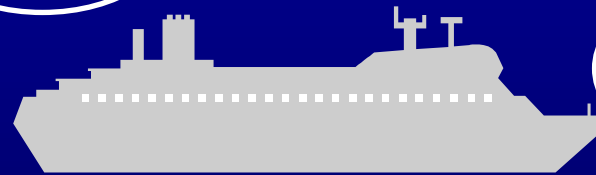
40% attack rate

Jan. 3-15

5.7% pax  
1.2% crew

17.8% pax  
6.2% crew

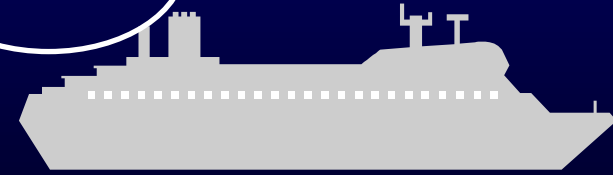
Jan. 15-29



Caribbean Itinerary

Jan. 7-22

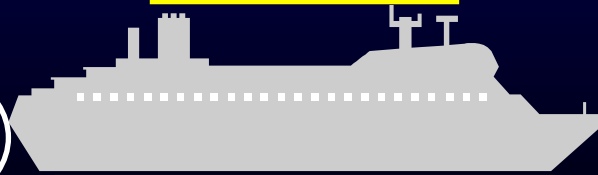
96% diarrhea  
46% vomiting



S. America Itinerary

Jan. 17-28

4.6% pax  
0.7% crew



Caribbean Itinerary

# 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

# Sequencing and Epidemiology

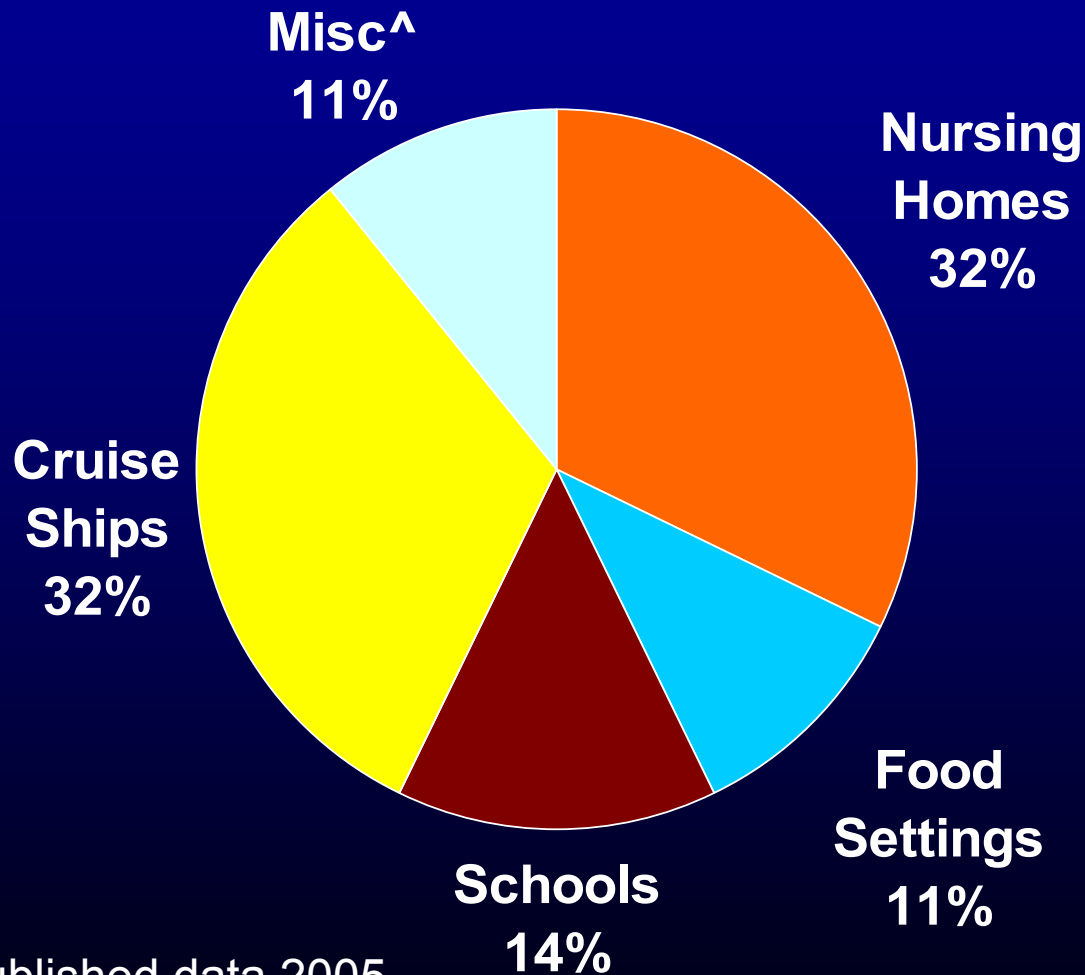
- Nucleotide sequencing analysis
  - ◆ Allows for the examination of emerging strains

# 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



# Farmington Hills strain by setting, 2002-2004 (N=28 outbreaks)\*



\*L. Blanton, Unpublished data 2005

^2 in communities, 1 beach

# 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)

# 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

# Conclusions

# Conclusions

- Norovirus leading cause of non-bacterial AGE in adults in land- and cruise ship-based outbreaks
- Transmitted by multiple routes
- Multiple strains co-circulate
- NoV is so common that we can not identify multi-state outbreaks without molecular epidemiology
- Better control and prevention by a better understanding of the epidemiology
- Future goals: Increased surveillance and better testing techniques through improved assays



# Acknowledgments



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- Susan Adams
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