Foodborne and waterborne diseases : a focus on viruses

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1. What is the situation in Europe and USA ?

USA : 310 10^6 inhabitants
9.3 10^6 km^2

Europe : 510 10^6 inhabitants
4.4 10^6 km^2

Outbreak : > 2 of similar cases exposure setting or food.

Evaluation of foodborne and waterborne diseases need a surveillance system

- USA

New surveillance system since 2009 National Outbreak Reporting System (NORS) for acute gastrenteritis (AGE)

- Europe

2005 in Stockolm (Sweden)

2002 in Parma (Italy)

Surveillance reporting since 2007

NORS provide surveillance on AGE*: it is very frequent (179 millions/year in USA : diarrhea or vomiting).

Acute Gastroenteritis Surveillance through the National Outbreak Reporting System, United States

Aron J. Hall, Mary E. Wikswo, Karunya Manikonda, Virginia A. Roberts, Jonathan S. Yoder, and L. Hannah Gould

Implemented in 2009, the National Outbreak Reporting System provides surveillance for acute gastroenteritis outbreaks in the United States resulting from any transmission mode. Data from the first 2 years of surveillance highlight the predominant role of norovirus. The pathogen-specific transmission pathways and exposure settings identified can help inform prevention efforts.

21 millions of AGE* are due to noroviruses (Vinje FEV congress 2012)

Surveillance based on internet-based system for health departments

Foodborne and waterborne diseases can be analyzed…

Hall *et al.* 2013

*AGE : acute gastroenteritis

Norovirus is the leading cause of reported AGE outbreaks in USA but also the leading cause of AGE hospitalizations and deaths

Table 1. Numbers of acute gastroenteritis outbreaks and outbreak-associated outcomes caused by various etiologic agents, National Outbreak Reporting System, United States, 2009–2010*

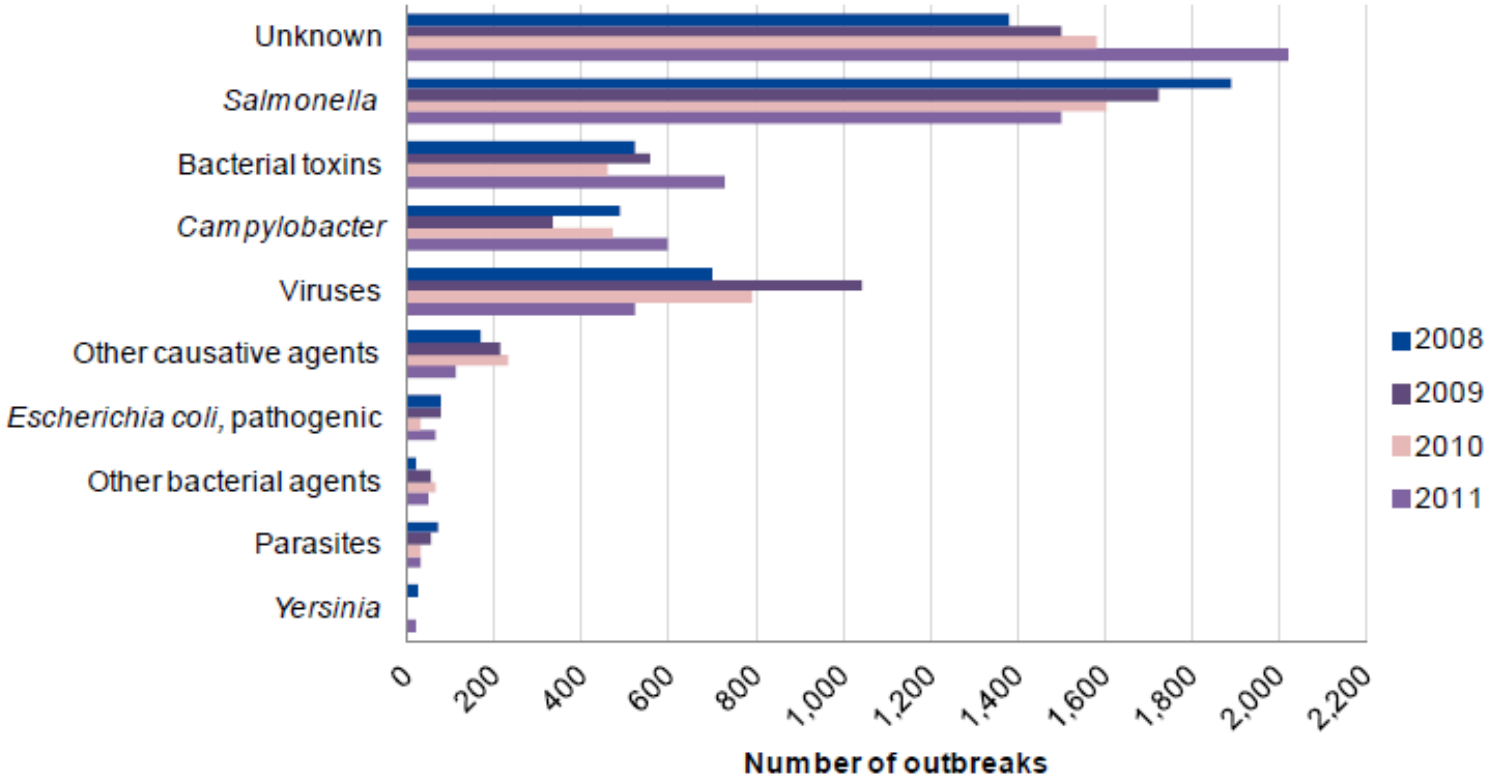
	No. (%) outbreaks			No. (%) outbreak-associated outcomes		
Outbreak etiology	Confirmed	Suspected	Total	Illnesses	Hospitalizations	Deaths
Single agent†						
Norovirus‡	1,355 (64.2)	553 (78.1)	1,908 (67.7)	69,145 (77.7)	1,093 (45.9)	125 (85.6)
Salmonella spp.	344 (16.3)	11 (1.6)	355 (12.6)	8,590 (9.7)	773 (32.5)	6 (4.1)
Shigella spp.§	99 (4.7)	10 (1.4)	109 (3.9)	2,135 (2.4)	115 (4.8)	1 (0.7)
STEC	88 (4.2)	13 (1.8)	101 (3.6)	1,091 (1.2)	250 (10.5)	9 (6.2)
Campylobacter spp.¶	56 (2.7)	13 (1.8)	69 (2.4)	1,550 (1.7)	52 (2.2)	0
Clostridium spp.#	41 (1.9)	21 (3.0)	62 (2.2)	3,242 (3.6)	16 (0.7)	3 (2.1)
Cryptosporidium spp.**	17 (0.8)	30 (4.2)	47 (1.7)	598 (0.7)	21 (0.9)	1 (0.7)
Bacillus spp.††	13 (0.6)	12 (1.7)	25 (0.9)	522 (0.6)	3 (0.1)	0
Staphylococcus aureus	11 (0.5)	11 (1.6)	22 (0.8)	263 (0.3)	0	0
Giardia intestinalis	13 (0.6)	6 (0.8)	19 (0.7)	121 (0.1)	5 (0.2)	0
Scombroid toxin/histamine	18 (0.9)	0	18 (0.6)	76 (0.1)	0	0
Ciguatoxin	14 (0.7)	0	14 (0.5)	59 (0.1)	6 (0.3)	0
Rotavirus	9 (0.4)	5 (0.7)	14 (0.5)	372 (0.4)	9 (0.4)	0
Other‡‡	33 (1.6)	23 (3.2)	56 (2.0)	1,194 (1.3)	38 (1.6)	1 (0.7)
All single-agent etiologies	2,111 (98.9)	708 (31.6)	2,819 (64.4)	88,958 (72.6)	2,381 (80.7)	146 (86.9)
Multiple agents	24 (1.1)	9 (0.4)	33 (0.8)	1,236 (1.0)	61 (2.1)	2 (1.2)
Unknown agent	0	1,524 (68.0)	1,524 (34.8)	32,294 (26.4)	510 (17.3)	20 (11.9)
All outbreaks	2,135 (100.0)	2,241 (100.0)	4,376 (100.0)	122,488 (100.0)	2,952 (100.0)	168 (100.0)

*STEC, Shiga toxin–producing *Escherichia coli*.
†Percentages for specific single agents are those among all single-agent etiology outbreaks (N = 2,819).
‡A norovirus genogroup was provided for 1,160 outbreaks: 150 GI, 1,003 GII, and 7 GI/GII.
§*S. sonnei* (95 confirmed and 8 suspected outbreaks), *S. flexneri* (5 confirmed outbreaks), *Shigella* sp. not known (1 confirmed outbreak).
¶*C. jejuni* (55 confirmed and 4 suspected outbreaks), *Campylobacter* sp. not known (8 confirmed and 2 suspected outbreaks).
#*C. perfringens* (37 confirmed and 20 suspected outbreaks), *Clostridium* sp. not known (4 confirmed and 1 suspected outbreak).
**C. parvum* (10 confirmed and 1 suspected outbreak), *C. hominis* (6 confirmed outbreaks), *Cryptosporidium* sp. not known (30 confirmed outbreaks).
††*B. cereus* (13 confirmed and 11 suspected outbreaks), *Bacilllus* sp. not known (1 suspected outbreak).
‡‡Includes *Vibrio* sp. (8 outbreaks), cyanobacterial toxins (6 outbreaks), enterotoxigenic and enteropathogenic *E. coli* (4 outbreaks), *Enterococcus* spp. (3 outbreaks), mycotoxins (3 outbreaks), *Cyclospora* spp. (2 outbreaks), pesticides (2 outbreaks), sapovirus (2 outbreaks), paralytic shellfish poison (1 outbreak), *Pseudomonas* sp. (1 outbreak), sodium hydroxide (1 outbreak), *Yersinia* sp. (1 outbreak), and other unspecified etiologies (22 outbreaks).

Hall *et al.* 2013

Viruses are the 2nd cause in Europe after *Salmonella*

Figure OUT4. Distribution of all food-borne outbreaks per causative agent in the EU, 2008-2011



Note: Food-borne viruses include calicivirus, hepatitis A virus and other unspecified food-borne viruses. Bacterial toxins include toxins produced by *Bacillus*, *Clostridium* and *Staphylococcus*. Other causative agents include mushroom toxins, marine biotoxins, histamine, mycotoxins, escolar fish (wax esters) and other unspecified agents. Parasites include primarily *Trichinella*, but also *Giardia*, *Cryptosporidium* and *Anisakis*. Other bacterial agents include *Listeria*, *Shigella* and *Brucella*.

2011 : 5648 outbreaks, 69553 cases, 7125 hospitalizations, 93 deaths

11 to 15 outbreaks/year are waterborne

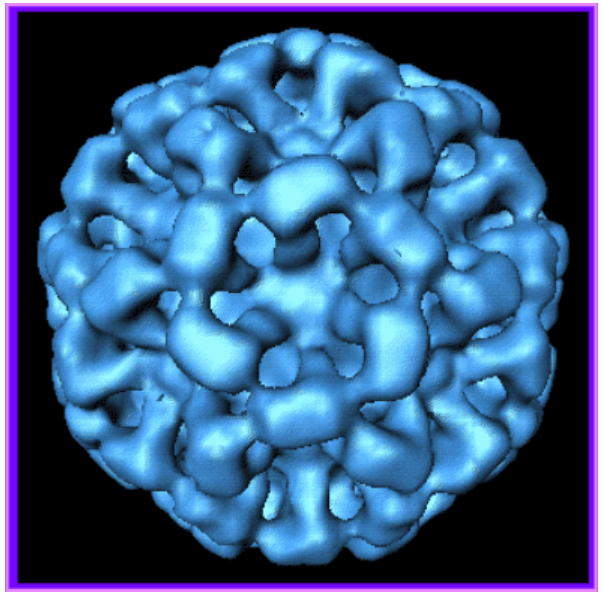
EFSA Journal 2013

2. Some informations on noroviruses also called :

Cruise ship virus

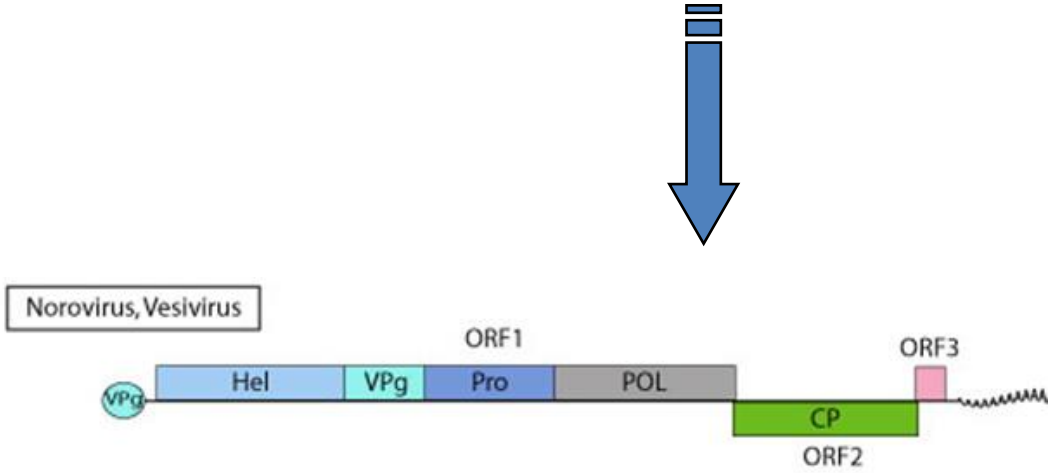
Winter vomiting virus

Norovirus



Structure of Norwalk virus at 3 angstroms resolution using X-ray crystallography (Dr Prasad)

Capsid 28-32 nm
with
ARNss (7,5kb)



http://expasy.org/viralzone/all_by_species/32.html

- First norovirus was isolated in 1972 (Kapikian)

- *Caliciviridae* family*, Norovirus* genus *with* 5 genogroups : I à V

- Human genogroup: I, II (swine) and less frequently IV

-Pathology

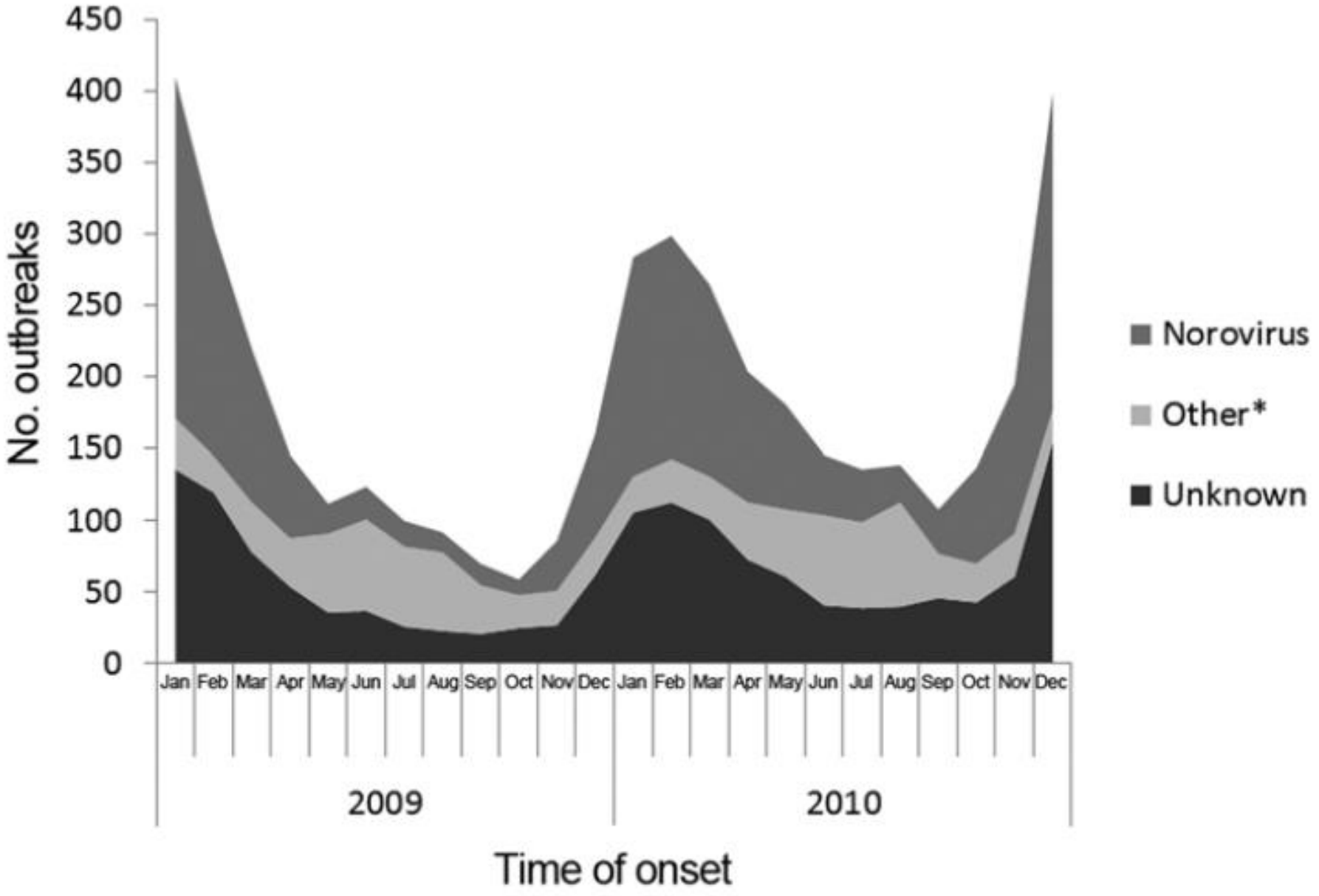
Incubation 24-48h

Symptoms (24-60 h): diarrhea (87%) ,
vomiting(74%)

and

abdominal pains (51%),
cramps (44%),
nausea (49%),
fever (32-45%).

AGE outbreaks exhibited winter seasonality in USA



Hall *et al.* 2013

The winter/spring pic in USA and Europe

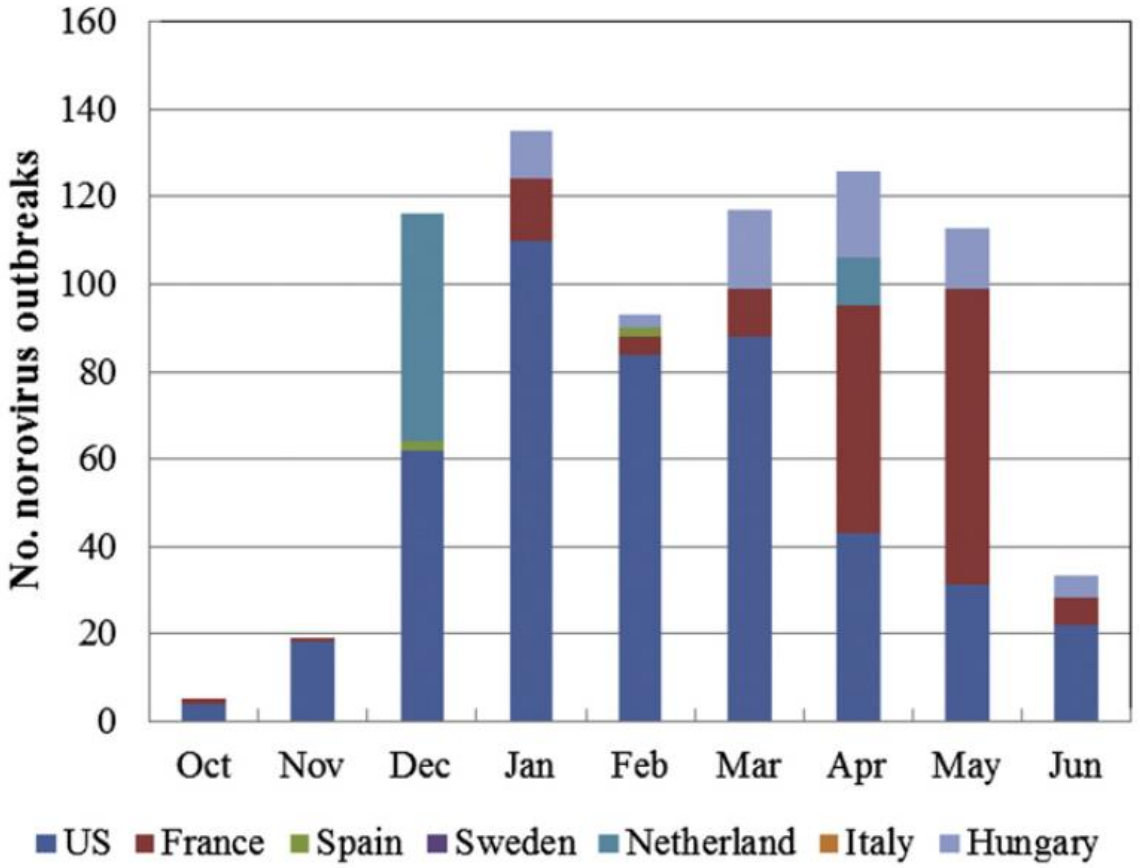


Fig. 1. Norovirus outbreaks linked to consumption of oysters in the Unites States (US), France, Spain, Sweden, Netherlands, Italy, and Hungary from October 2009 to June 2010. The European data are collected from the Foodborne Viruses in Europe network (FBVE) (http://www.noronet.nl/fbve/databases/) while the US data are from Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/).

Wang and Deng (2012)

- Norovirus are not cultivable

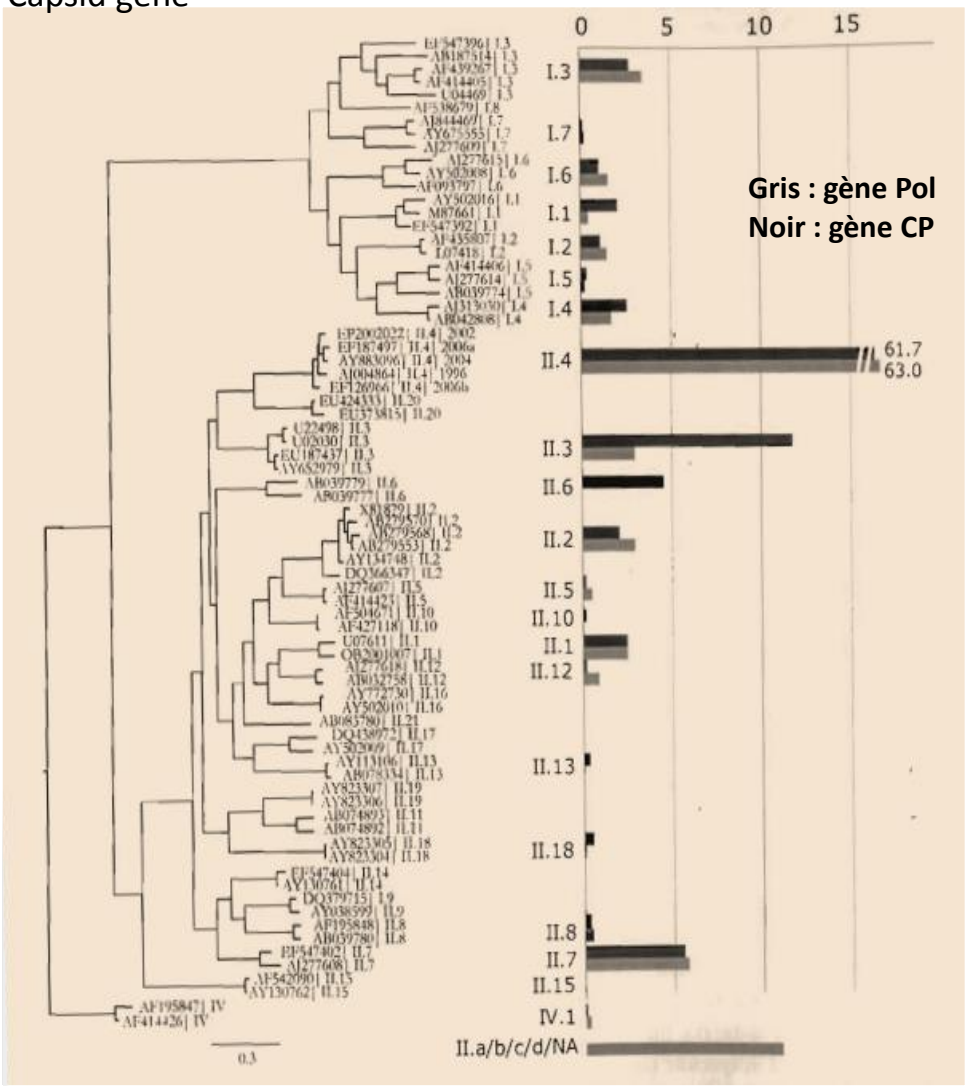
- No long term immunity

- High genetic variability

-Genotype GII.4 is the most prevalent

Capsid gene

Pourcentage of all cases from 1990 to 2008



Siebenga *et al.* 2009

Periodic emergence of Norovirus variants

JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2006, p. 327–333
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.2.327–333.2006

Vol. 44, No. 2

Emergence of a New Norovirus Genotype II.4 Variant Associated with Global Outbreaks of Gastroenteritis

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Norovirus (NoV) is highly infectious and is the major cause of outbreak gastroenteritis in adults, with pandemic spread of the virus being reported in 1995 and 2002. The NoV genome is genetically diverse, which has hampered development of sensitive molecular biology-based methods. In this study we report on a nested reverse transcriptase PCR (nRT-PCR) that was designed to amplify the highly conserved 3′ end of the polymerase region and the 5′ end of the capsid gene of NoV genogroup II (GII). The nRT-PCR was validated with strains isolated from sporadic and outbreak cases between 1997 and 2004 in New South Wales, Australia. Phylogenetic analysis identified six genotypes circulating in New South Wales, GII.1, GII.3, GII.4, GII.6, GII.7, and GII.10, with GII.4 being the predominant genotype. In 2004, there was a marked increase in NoV GII activity in Australia, with a novel GII.4 variant being identified as the etiological agent in 18 outbreaks investigated. This novel GII.4 variant, termed Hunter virus, differed by more than 5% at the amino acid level across the capsid from any other NoV strain in the GenBank and EMBL databases. The Hunter virus was subsequently identified as the etiological agent in large epidemics of gastroenteritis in The Netherlands, Japan, and Taiwan in 2004 and 2005.

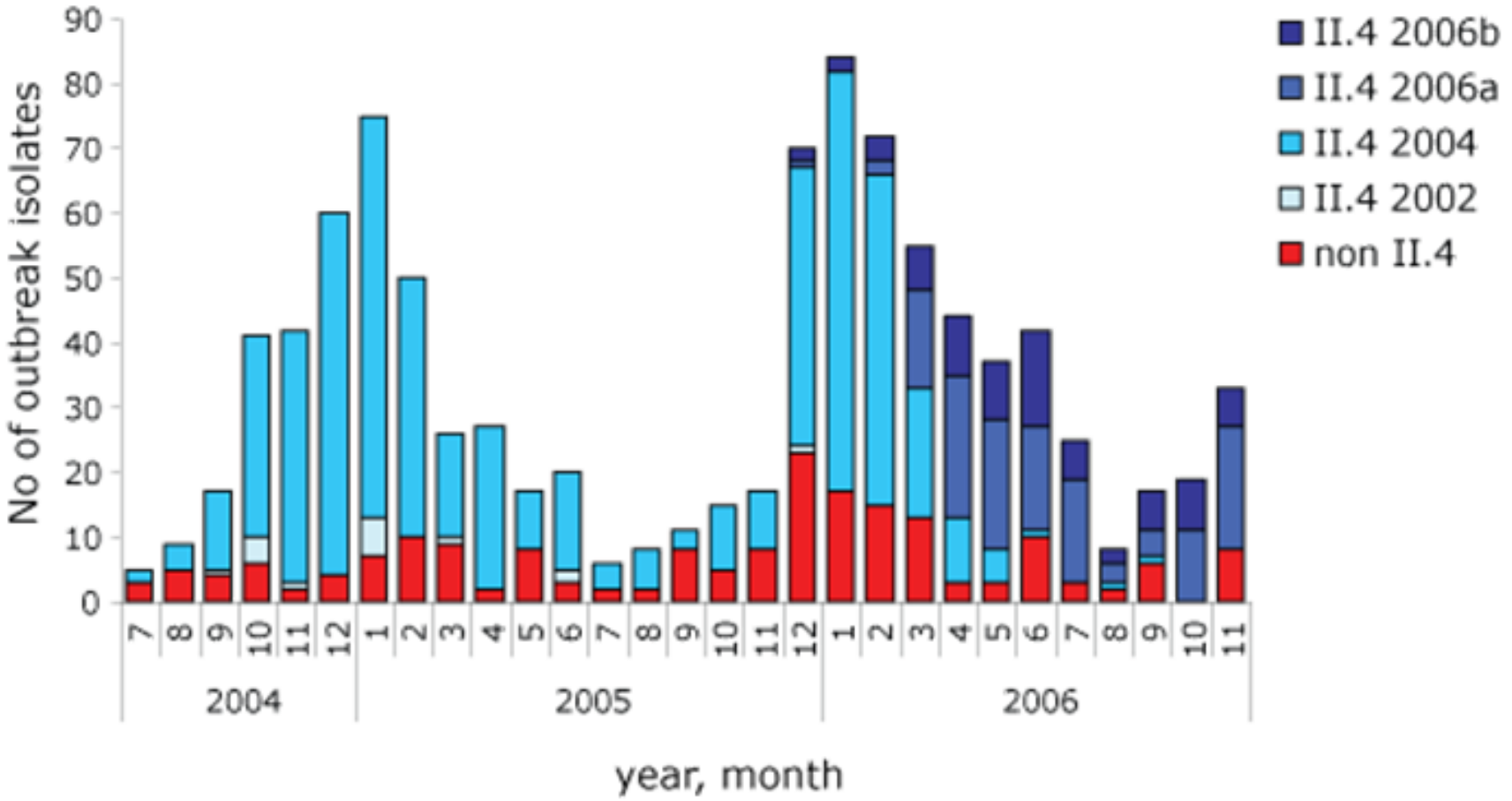
18 outbreaks in Australia 2004 : new variant GII.4 = Hunter virus

5% differences in a.a. sequence (capsid)

The Netherlands, Japan, Taiwan

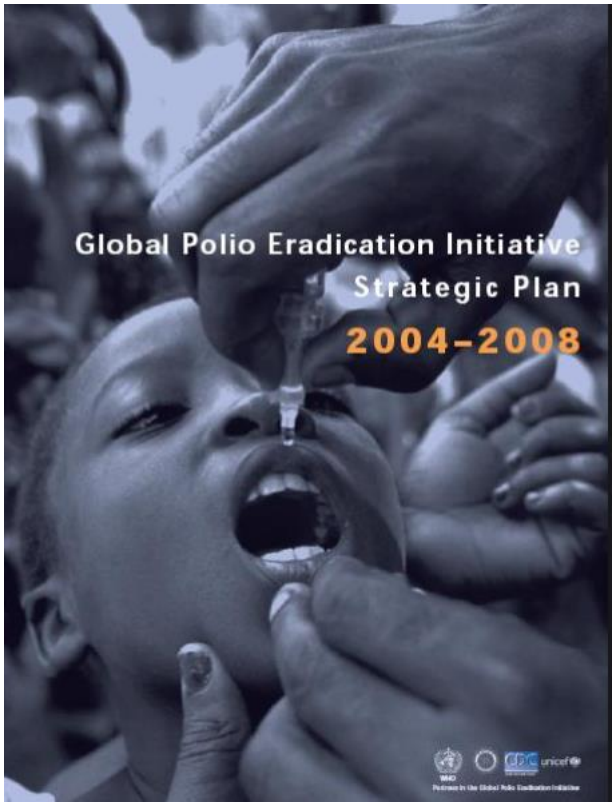
Bull *et al. (*2006)

They may provoke pandemics : ex for GII.4

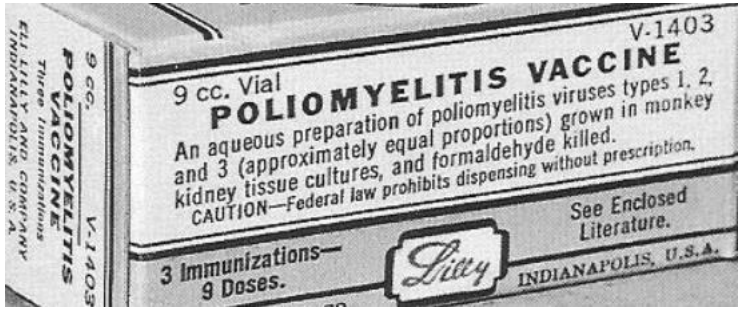


Kroneman *et al.* (2006)

Don’t forget other foodborne and waterborne viruses : enteric viruses

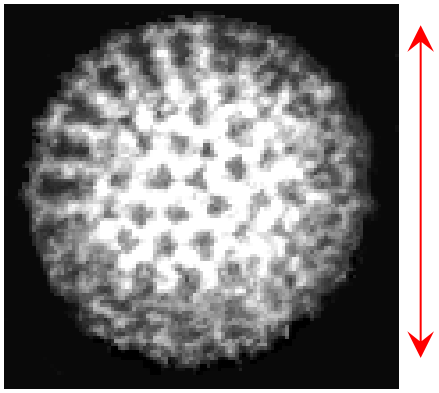


Poliovirus was a very efficient waterborne pathogen

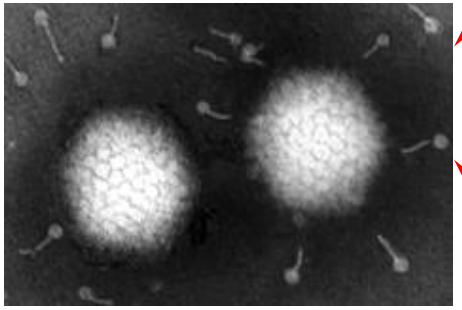


The other foodborne and waterborne viruses

Gastroenteritis virus



70-80 nm



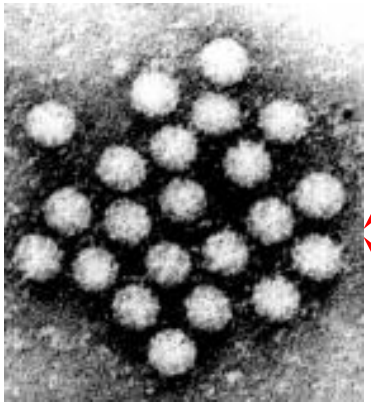
70-80 nm

*Rotavirus (*ARNds*)*

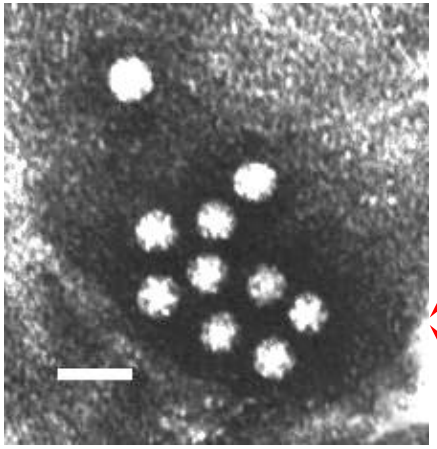
http://pathmicro.med.sc.edu/mhunt/RNA-HO.htm

Adenovirus 40 and 41 (ADNds)

http://www.worsleyschool.net/science/files/virus/page.html



28-32 nm



20-30 nm

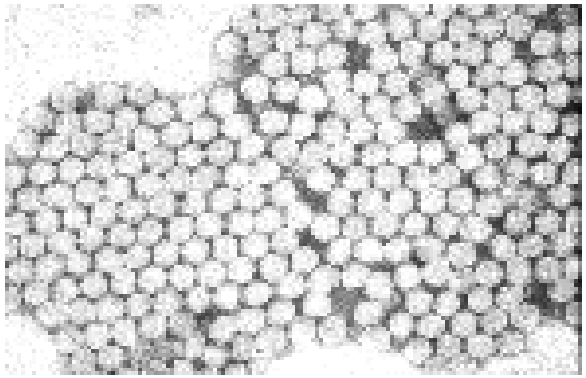
Norovirus and *Sapovirus* (ARNss)

http://virology-online.com/viruses/Diarrhoea5.htm

*Astrovirus (*ARNss*)*

http://www.stanford.edu/group/virus/astro/2004ambili/Astrohome.html

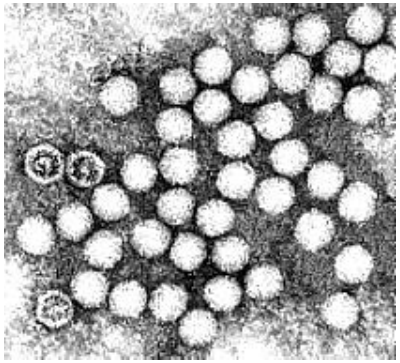
Hepatitis viruses



Hepatitis A and E virus

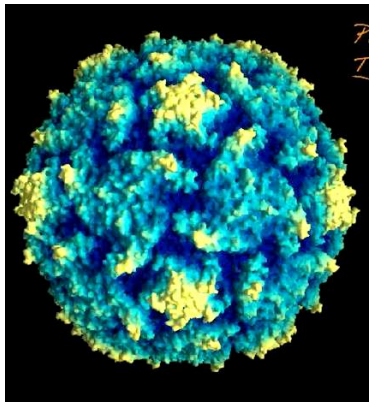
http://www.scottremley.com/viral_images/Hepatitis_A_Virus_Color.gif

Enterovirus



Coxsackievirus A et B, *Echovirus*...

http://www.worsleyschool.net/science/files/virus/page.html



20-30 nm (ARNss)

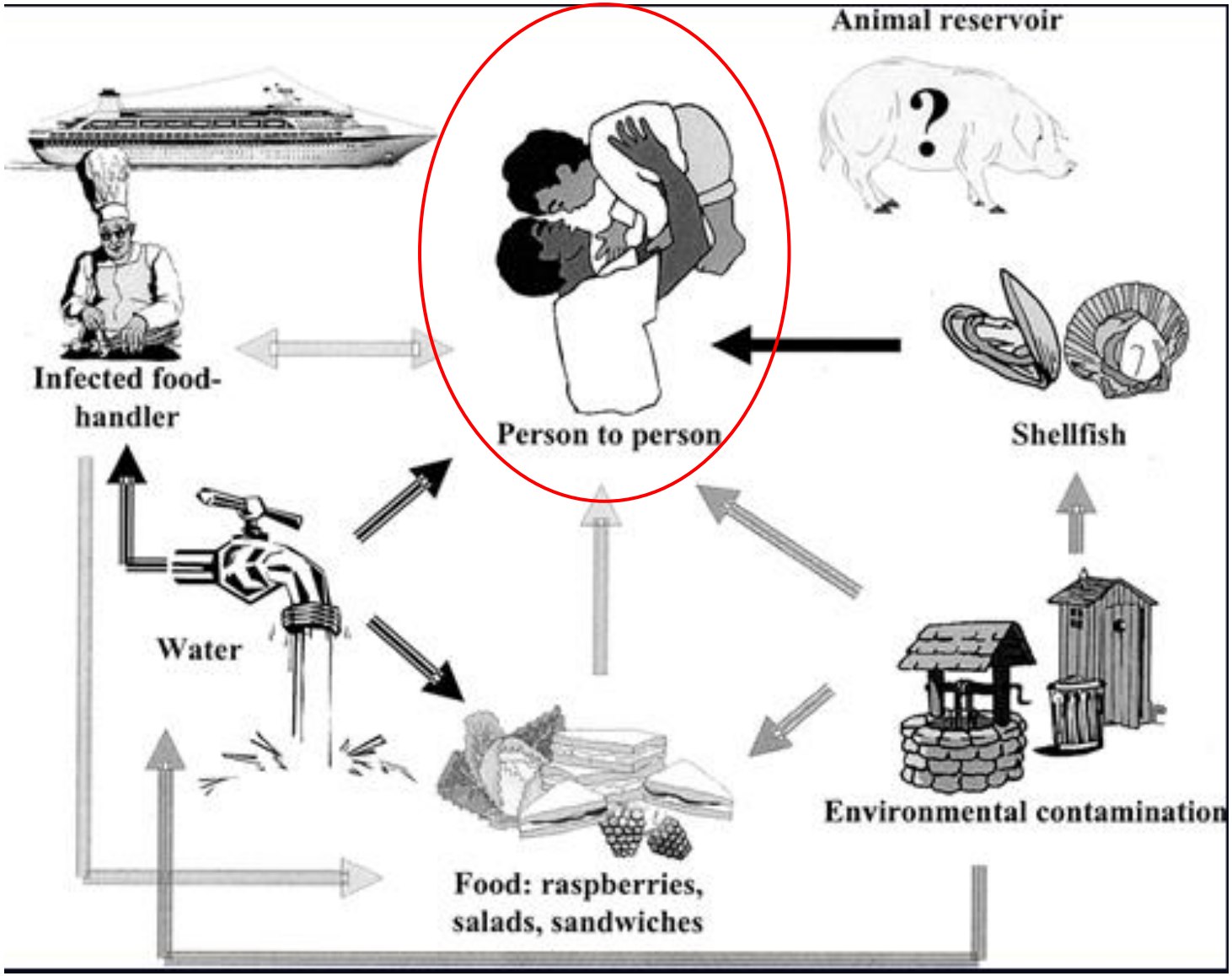
http://www.madrimasd.org/blogs/salud_publica/2008/03/28/87621

Viral excretion in human stools

	Quantity of virus /g of stool	Excretion duration
Hepatitis A and E virus	10^9	14-21 days
Gastroenteritis virus	10^8-10^{10}	3-12 days
Enterovirus	10^3 - 10^6	20-30 days

3. Way of transmission : the fecal oral route

Way of transmission



http://www.cruiselawnews.com/2010/03/articles/norovirus/celebritys-mercury-cruise-ship-free-of-norovirus/

Person to person transmission is the main route :

European surveillance 88% (Kroneman *et al.* 2008)

High transmission capacity (number of secondary cases from one case : R0)

R0 = 14 (without any hygiene)

R0 = 2 (good hygiene procedures)

Pandemic flu in 1918 : R0 = 2-3

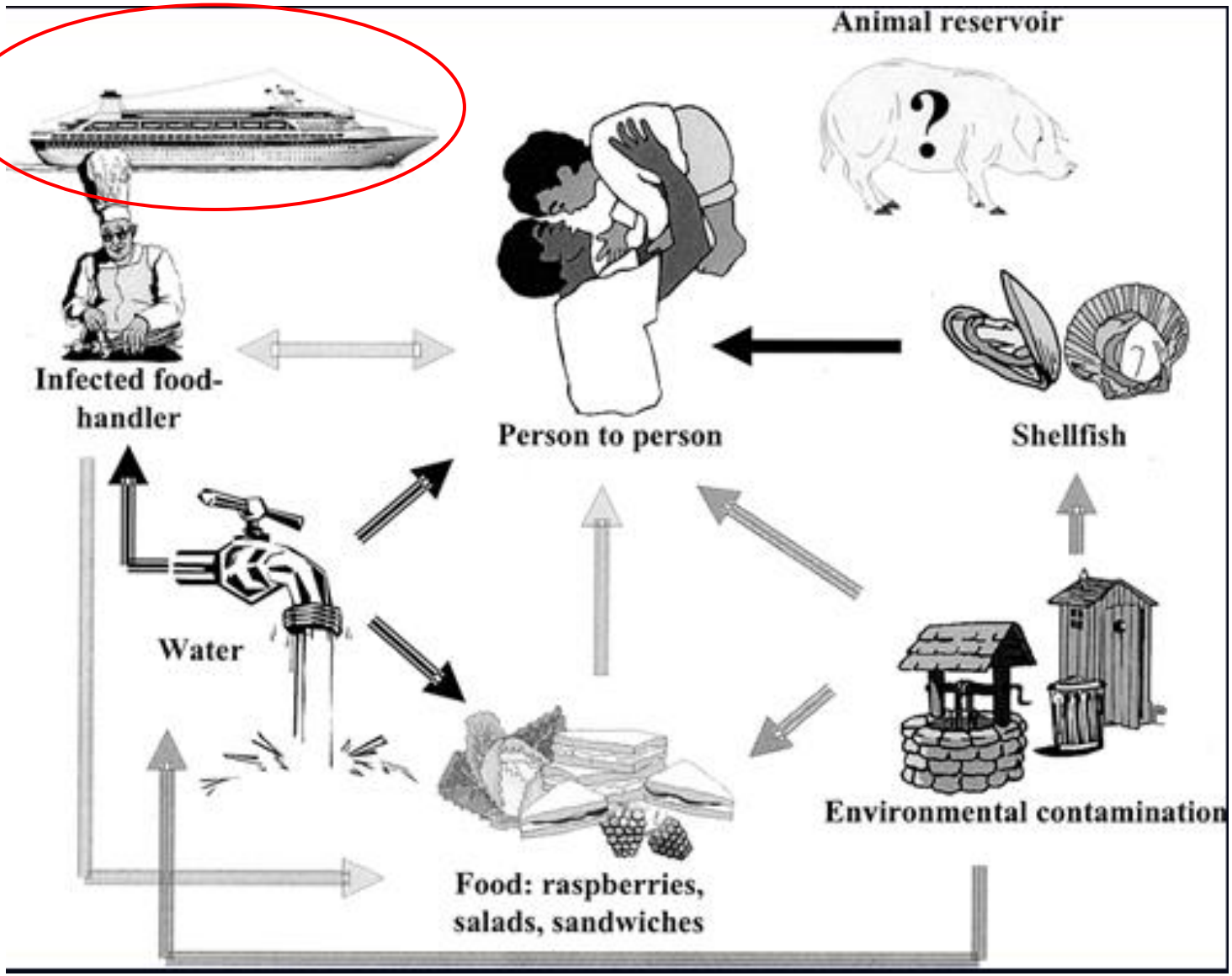
Poliovirus : R0 = 5-7

Why ?

- High excretion
- Low infective dose (DI_{50} = 20 viral particles)
  - 1g stools may infect 5 10^8 persons
  - Vomiting once may infect 3 10^5 to 3 10^8 persons
- No long term immunity
- High resistance in environment

CDC, 2009 ; Mills et al. 2004 ; Heijne et al. 2009 ; Teunis et al. 2008 ; Joukje et al. 2010

Way of transmission



http://www.cruiselawnews.com/2010/03/articles/norovirus/celebritys-mercury-cruise-ship-free-of-norovirus/

Confined setting favor person to person transmission: Cruise ship

3 confirmed outbreaks in March 2013

Update: Cruise Ship Norovirus Woes Continue with Three Confirmed Outbreaks in March

(9:30 a.m. EDT) -- The month of March has seen an uptick in the number of gastrointestinal illnesses reported on cruise ships.

According to the U.S. Centers for Disease Control and Prevention Web site, an outbreak on Royal Caribbean's **Vision of the Seas** affected 121 out of 2,756 passengers and crew on an 11-night Caribbean sailing that ended March 8. A second outbreak in March, on Princess Cruises' **Ruby Princess**, affected over six percent of passengers and crew -- or 276 out of a total ship population of 4,300 -- on a 7-night Caribbean cruise.

Most recently, 84 passengers reported symptoms over the course of 21 days onboard Cunard's **Queen Elizabeth**.

Local Los Angeles news station **KTLA 5** quoted **Cunard** as saying four percent of the 1,900 passengers on the five-week tour of the South Pacific became ill, roughly the same percentage as onboard Vision of the Seas. A spokesperson for Cunard told Cruise Critic that by the time the ship reached port in Los Angeles no passengers were reporting symptoms. And, after a thorough cleaning of the ship while in port, only one passenger onboard has reported symptoms since. The ship has filed a report with the CDC, however.

Cruise lines participating in the CDC's Vessel Sanitation Program -- and every major operator does -- are required to report the total number of GI cases evaluated by the medical staff before the ship arrives at a U.S. port when sailing from a foreign port. A separate notification is required when the count exceeds 2 percent of the total number of passengers or crew onboard. The CDC mandates a standardized protocol of reporting and cleaning procedures during and following an outbreak onboard. The organization then completes its own investigation to attempt to determine the cause of the illness.

N= 121/2756

N= 276/4300

N=84

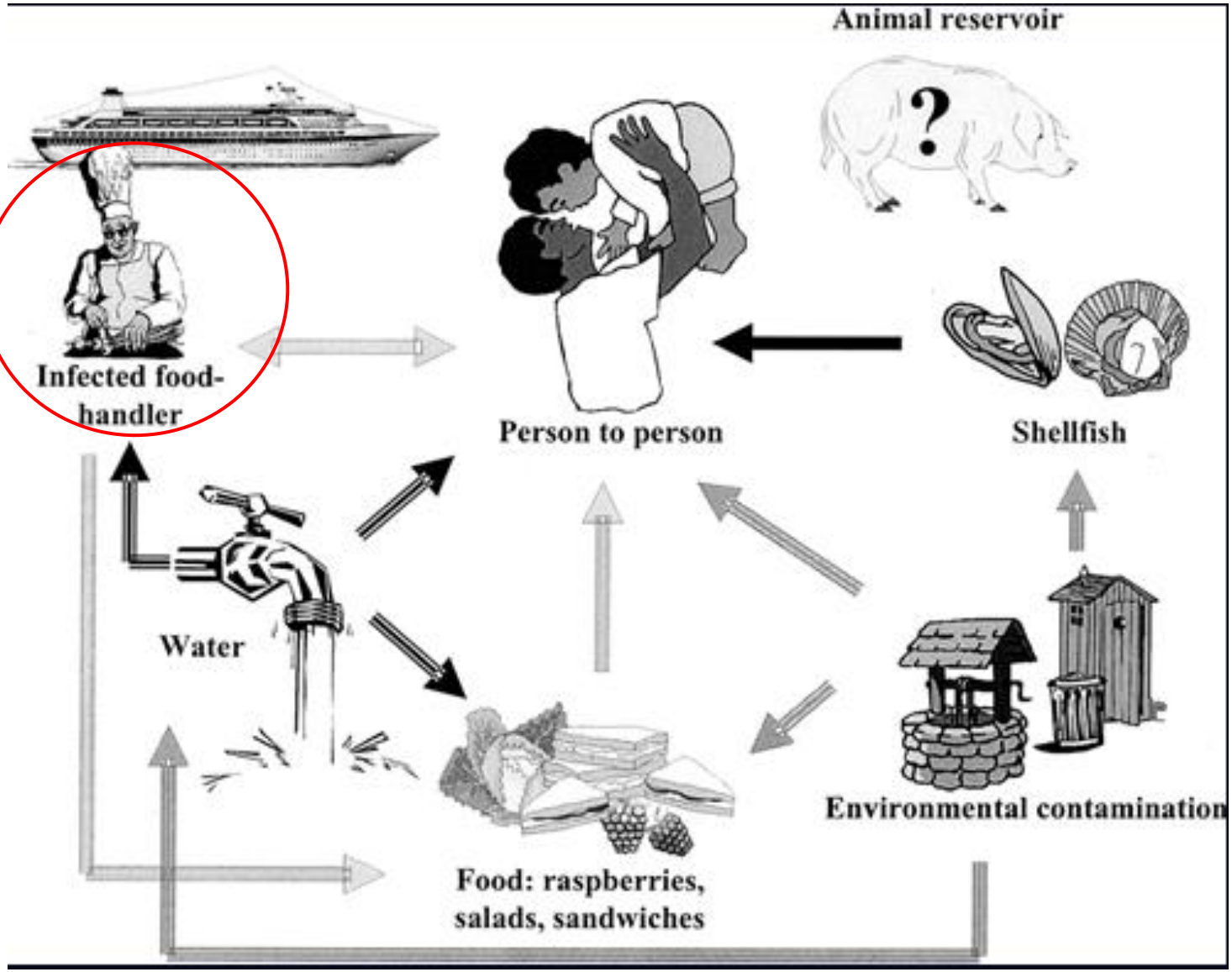
Special procedure for cleaning (CDC)

http://www.cruisecritic.com/news/news.cfm?ID=5255

Also hospitals, nursing homes, elderly homes…

Elderly homes : 71% due to noroviruses (Greig *et al.* 2009)

Way of transmission



http://www.cruiselawnews.com/2010/03/articles/norovirus/celebritys-mercury-cruise-ship-free-of-norovirus/

Hygiene is very important : infected person may contaminated food

Large outbreak of norovirus: The baker who should have known better

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KEYWORD
Norovirus

Summary *Introduction:* In January 2001, 231 persons from the staff of a department in the Netherlands fell sick with diarrhoea and vomiting after a buffet lunch, which was prepared and served at a restaurant. Eighteen restaurant employees also reported illness.
Materials and methods: To determine risk factors for illness a questionnaire was e-mailed to department staff and returned electronically. Employees from the restaurant and the bakery supplying the rolls were interviewed. Stool samples were collected from reported cases and from all the staff of the restaurant and the bakery supplying the rolls. Stools were tested for bacteria and noroviruses.
Results: Analyses of the questionnaires showed an increasing risk of illness with the number of rolls eaten (OR = 2.0 95%CI = 1.5–2.5).

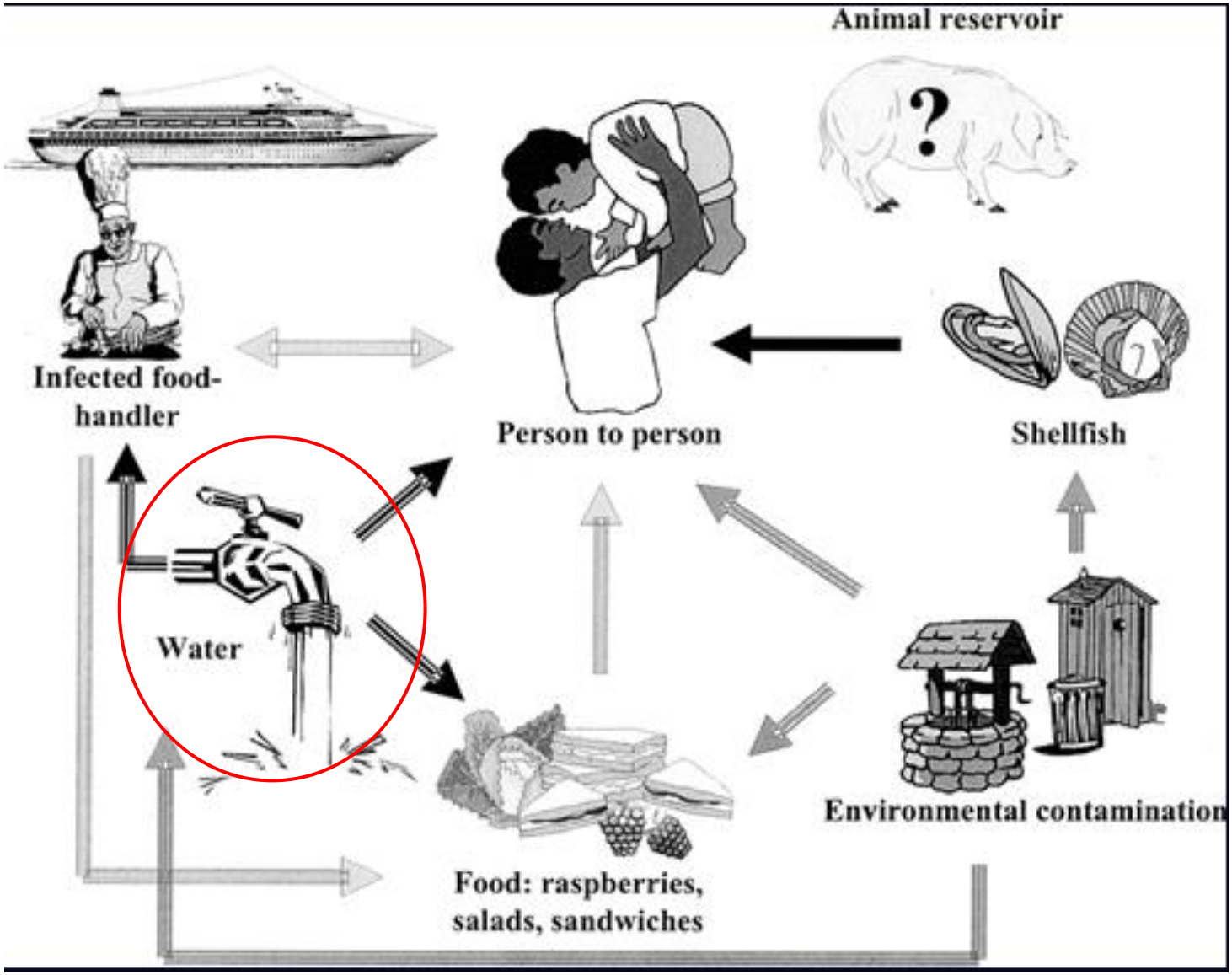
Investigations revealed the baker was suffering from gastroenteritis and had vomited in the bakery sink the day he prepared the rolls. However, he had cleaned up and washed his hands before continuing to work. Norovirus with an identical sequence was detected in the stool samples of ill persons from the department, and symptomatic employees from the restaurant and the bakery.
Conclusion: Foodhandlers are unaware of the potential for transmission of norovirus. Use of electronically mailed questionnaires allowed rapid gathering and analysis of a large amount of data and subsequent identification of the source when detection of virus from the source (the baker) was still possible.

N= 231

Rolls contaminated by the baker

Wit *et al.* 2007

Way of transmission



http://www.cruiselawnews.com/2010/03/articles/norovirus/celebritys-mercury-cruise-ship-free-of-norovirus/

It is usually difficult to demonstrate the link between AGE and drinking water : possibilities for high numbers of infections

Waterborne gastroenteritis outbreak at a scouting camp caused by two norovirus genogroups: GI and GII

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ABSTRACT

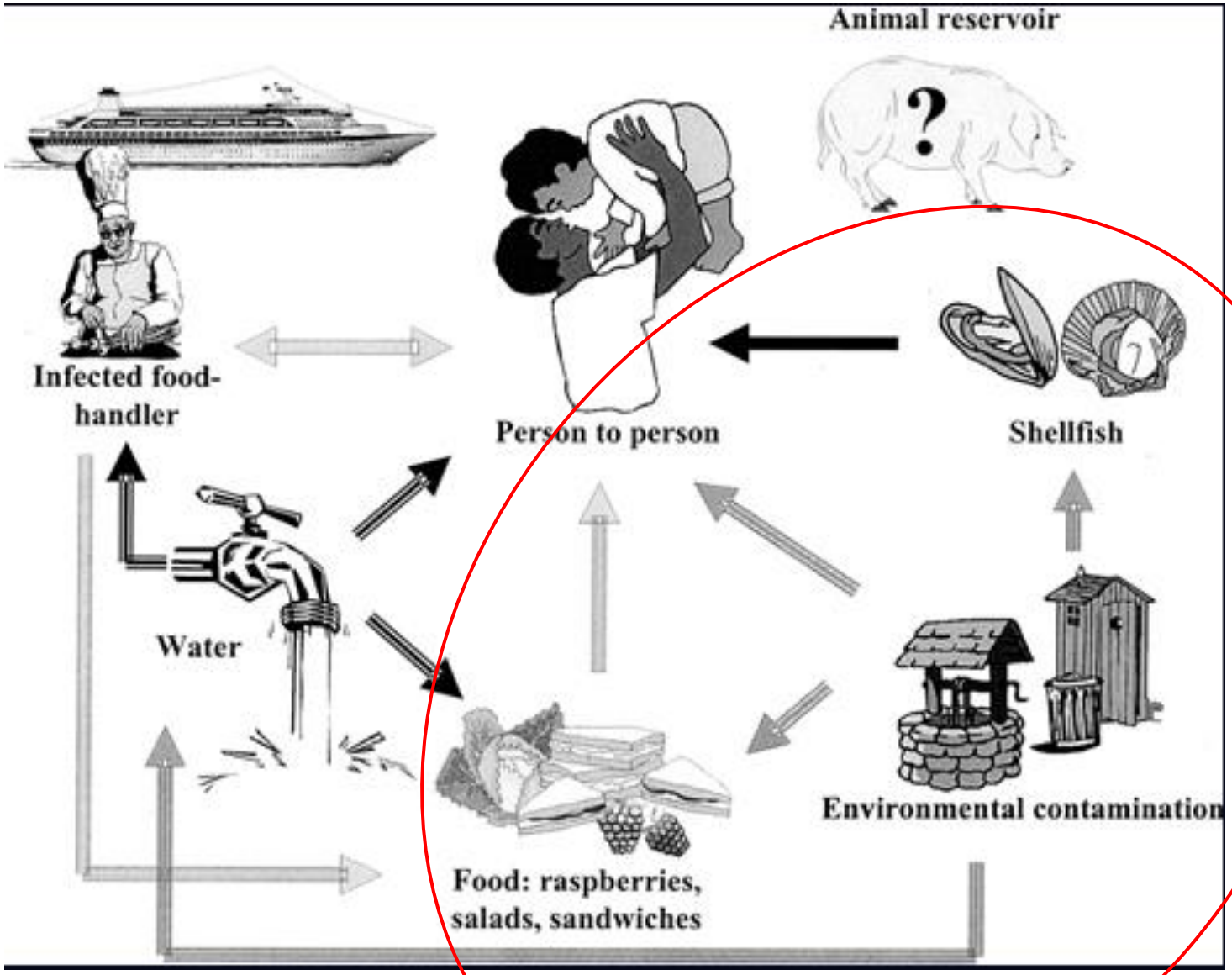
Background: A cross-border gastroenteritis outbreak at a scouting camp was associated with drinking water from a farmer's well.
Objectives: A retrospective cohort study was performed to identify size and source of the outbreak, as well as other characteristics.
Study design: Epidemiological investigation included standardized questionnaires about sex, age, risk exposures, illness and family members. Stool and water (100 mL) samples were analyzed for bacteria, viruses and parasites.
Results: Questionnaires were returned by 84 scouts (response rate 82%), mean age of 13 years. The primary attack rate was 85% (diarrhoea and/or vomiting). Drinking water was the strongest independent risk factor showing a dose–response effect with 50%, 75%, 75%, 93% and 96% case prevalence for 0, 1, 2–3, 4–5 and >5 glasses consumed, respectively. Norovirus (GI.2 Southampton and GII.7 Leeds) was detected in 51 stool specimens (75%) from ill scouts. Water analysis showed fecal contamination, but no norovirus. The secondary attack rate was 20%.
Conclusions: This remarkable outbreak was caused by a point-source infection with two genogroups of noroviruses most likely transmitted by drinking water from a well. Finding a dose–response relationship was striking. Specific measures to reduce the risk of waterborne diseases, outbreak investigation and a good international public health network are important.

Fecal contamination but no norovirus found in water

Waarbeek *et al.* 2013

Same for Arvelo *et al.* 2012 in Guatemala

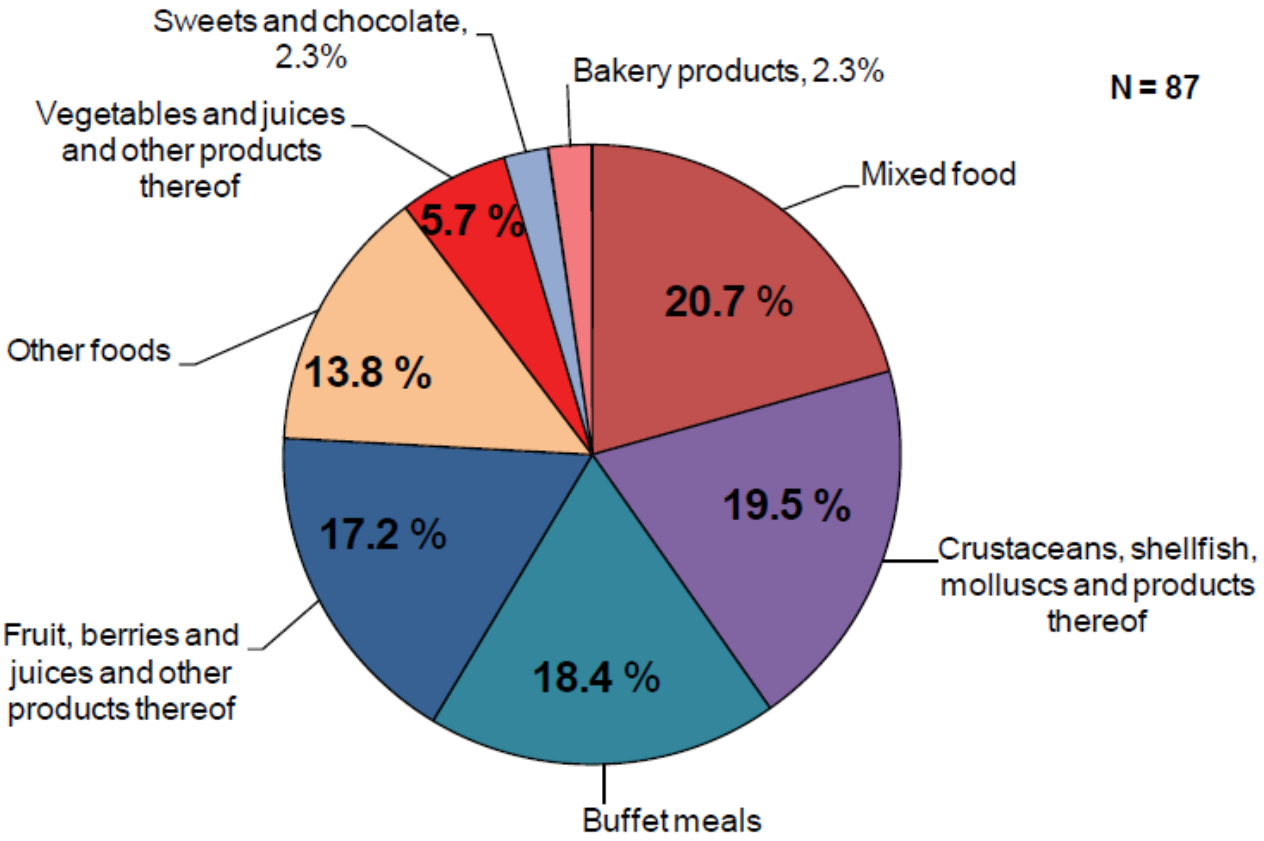
Way of transmission



http://www.cruiselawnews.com/2010/03/articles/norovirus/celebritys-mercury-cruise-ship-free-of-norovirus/

Food vehicule for noroviruses

Figure OUT21. Distribution of food vehicles in strong evidence outbreaks caused by caliciviruses, (excluding strong evidence waterborne outbreaks) in the EU, 2011



EFSA 2013

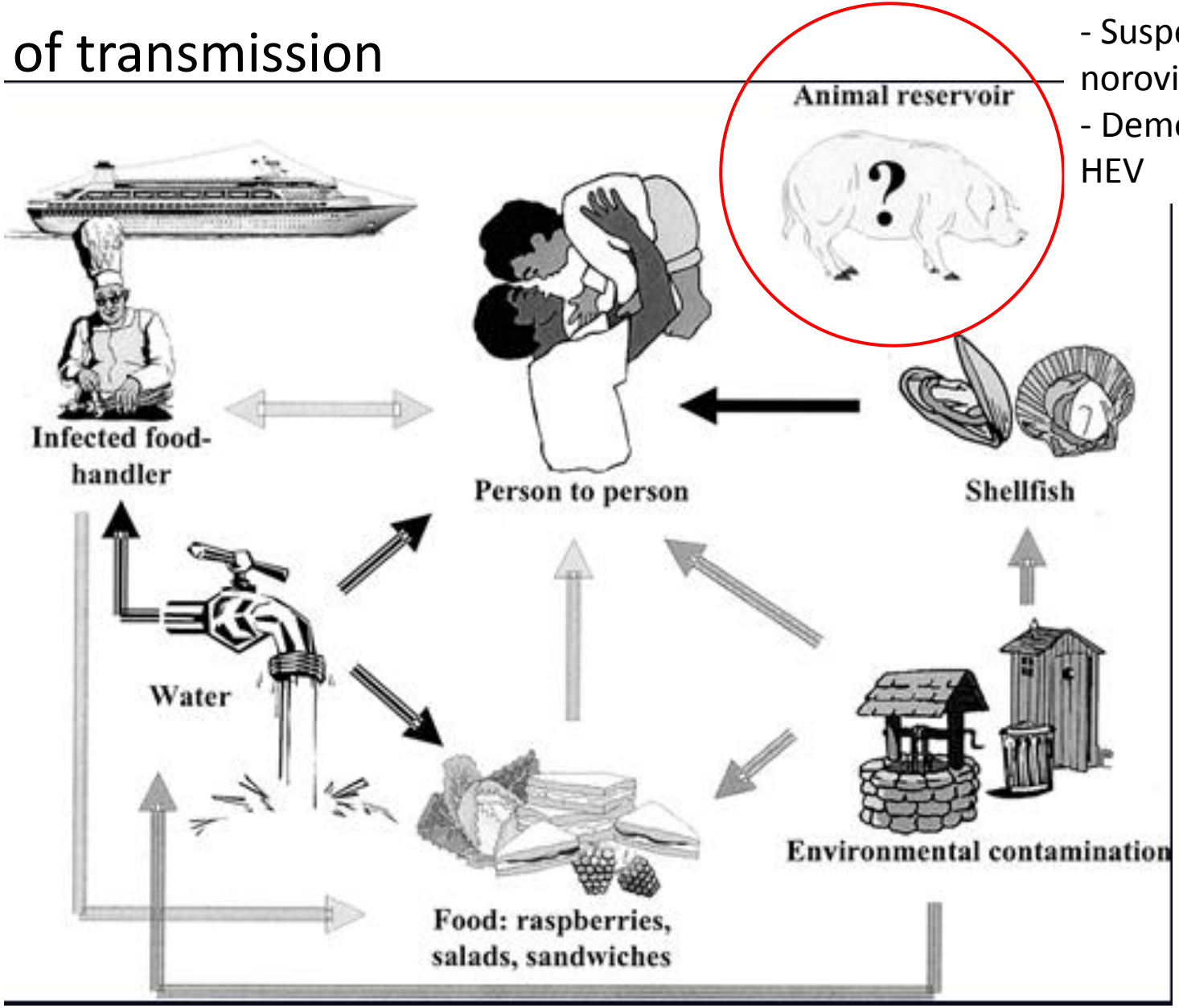
Note: Data from 87 outbreaks are included: Austria (1), Denmark (37), Finland (10), France (13), Germany (7), Hungary (2), Netherlands (2), Poland (1), Spain (3), Sweden (7) and United Kingdom (4).
Other foods (N = 12) include: bovine meat and products thereof (1), dairy products (1), eggs and egg products (1), fish and fish products (1), and other foods (8).

High increase for mixed food and berries…

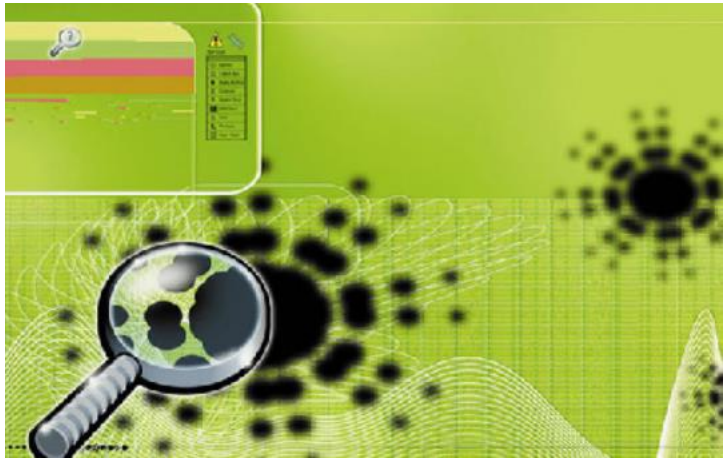
Environmental impact ?

Way of transmission

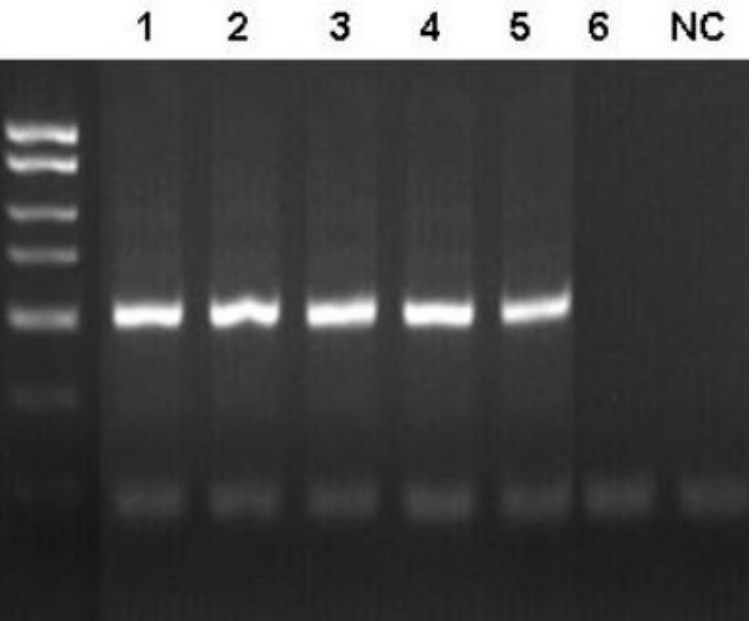
- Suspected for noroviruses
- Demonstrate for HEV



http://www.cruiselawnews.com/2010/03/articles/norovirus/celebritys-mercury-cruise-ship-free-of-norovirus/



4. Detection of viruses



Epidemiological criteria:

Verified vs possible

- Vomiting > 50%
- Incubation 24h-48h
- Duration 12-60h
- Absence of pathogenic bacteria

Sensitivity 68%
Specificity 99%

(Turcios *et al.* 2006)

Now strong evidence or weak evidence (EFSA 2013)

Different steps for detection

1. Concentration / Extraction

    - Adsorption /elution or ultrafiltration for liquid samples

    - Extraction or elution for solid food

2. Detection

    - RT-PCR

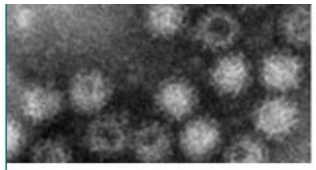
(quality control : inhibition and false positive or negative)

Standardization : CEN/TC275/WG6/TAG4 (Norovirus and HAV for water and food).

3. Identification : typing tool *Norovirus*

http://www.rivm.nl/mpf/norovirus/typingtool

Foodborne viruses in europe network



NoroNet

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> FBVE net

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  - Activities
  - Output
  - Databases
- Databases
- Members Noronet
- Members FBVE

FBVE net

FBVE Net

FBVE net is a network of virologists and epidemiologists in 13 European countries existing since 1999, sharing surveillance and research data on enteric virus infections, focusing mainly on norovirus.
The network maintains a shared database accessible via the Internet for data entry and sharing and analysis.

http://www.noronet.nl/noronet/

Prevalence of Norovirus genome is sometimes very high !

- Oyster (production zone)

  *76.2% (n= 844) (GB) (Lowther *et al.* 2012)

  60% > 100 cg/g ; 30% >1000cg/g some with 10 000cg/g !

  *3.9% (4.4% HAV) (n= 390) (USA) (DePaola *et al.* 2010)

  *9% to 23% NV (F) (Beuret *et al.* 2003 ; Le Guyader *et al.* 2000)
  *9% NV in marketed oysters (n=387) F (Schaeffer *et al.* 2013)
  * Other countries : from <1% to 52% (DePaola *et al.* 2010)

- Red fruits

  *7% et 34% France and Belgium (Baert *et al.* 2011)

- Salads *0,8% to 12,4% (n=210) (Adria Normandie – Prevavir 2011)

5. Preventing norovirus infections

- Good hygiene practices / HACCP :

Hygiene procedures for food handler

Avoid environment pollution. After the things may be more complicated (irrigation, oyster growing, drinking water ressource) !

Use treatments able to decrease viral pollution (wastewater, drinking water)

- Use the rigth indicators of viral/fecal pollution.

- Detect pathogenic viruses in some cases: control their absence, epidemiological studies…

Conclusions

The epidemiological data shows that viruses are :

- An important problem in USA and Europe

- Find everywhere over the world

- Food: Oysters/Raspberries/ Vegetables and fruits/sandwiches…

- Water : drinking water, irrigation or growing zones for oysters,

- Frequence : Norovirus, Hepatitis A virus, other virus…

- Problem for collectivities :
Nurse homes, hospital, elderly homes, restaurants, cruiseships…

- Costs are important :
Nosocomial outbreak, 355 infections : cost 650 000 $ (Johnston *et al.* 2007)