Viral Agents of Gastroenteritis: How Important are they?

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Director, Fogarty International Center
Associate Director for International Research, NIH

Dijon
June 14, 2007
Everyone Poops
Estimated global distribution of the 2.2 million annual childhood deaths caused by diarrhea

1 dot = 5,000 deaths

20% of deaths < 5 yrs
The Estimated Burden of Gastroenteritis in the United States

Episodes 267,000,000
OPD Visits 612,000
Hospitalizations 160,000 –(8-12%)
Deaths 6,200
Deaths 300
Children 2,000,000
Children 19,400,000

Total

CDC
The "Diagnostic Void"

Survey of >30,000 specimens from 10 hospitals

Unknown (94%)

Bacterial (5.9%)

Salmonella
Shigella
Campylobacter
E. coli

ref: L. Slutsker, 1997
Etiology of 7,458 Outbreaks of Gastroenteritis Reported to CDC, 1973-1987

- Unknown (62%)
- Bacterial (25%)
- Chemical (9%)
- Parasitic (1.8%)
- Viral (1.8%)

ref: Bean & Griffin, 1990
Viral Agents of Gastroenteritis

- Rotavirus
- Adenovirus
- Astrovirus
- Calicivirus - NLV
- Calicivirus - SLV
- Torovirus
- Picobirnavirus
- Enterovirus 22
Noroviruses (a.k.a. Norwalk-like Viruses, human caliciviruses)

- Identified by electron microscopy (Kapikian)-1972
- Do not grow in cell culture; no animal model
- Commercial detection assays under development
- Molecular detection by RT-PCR
- Many different genotypes

• Most common cause of outbreaks of nonbacterial acute gastroenteritis
Norovirus Detection by RT-PCR/EIA

Norovirus genome contains four protein-coding open reading frames (ORFs): ORF 1, ORF 2, ORF 3, and Capsid. ORF 1 encodes for Hel and Pro, ORF 2 for Pol, and ORF 3 for Capsid.

**CDC Primers**
- A: 123 bp
- B: 213 bp
- C: 322 bp

**Others**
- EU: 327 bp

**PCR Target**
- ORF 1
- ORF 2

**Expressed Antigen**
- 177/253 bp
- 123 bp
- 213 bp
- 322 bp

**Table**

<table>
<thead>
<tr>
<th>ORF 1</th>
<th>ORF 2</th>
<th>ORF 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1789</td>
<td>5358</td>
<td>6950</td>
</tr>
<tr>
<td>5374</td>
<td>530</td>
<td>7588</td>
</tr>
<tr>
<td>6950</td>
<td>212</td>
<td>7654</td>
</tr>
</tbody>
</table>
**Stool cup-to-Sequence**

Detection & Characterization of NLVs

- RNA extraction
- RT-PCR
- Purify Gel Band
- Automated sequencing
- Electrophoresis stained Gel
- Sequence Analysis
- Phylogenetic Analysis

**Flowchart:**
- RNA extraction → RT-PCR → Gel Band → Automated sequencing → Sequence Analysis → Phylogenetic Analysis → Electron Microscopy
The New York Times

FRIDAY, JANUARY 25, 1991

IS OUR FISH FIT TO EAT?

A six-month investigation of fresh fish and shellfish raises serious questions about their quality.

By Dana Balart

Capital News Service

BALTIMORE — Gov. William Donald Schaefer Friday suggested that Maryland would consider a "stay" for the following harvesting season, pending further research, on the fish and shellfish sold to Maryland residents and visitors.

Schaefer made his remarks during a news conference in the state house. He said the state had received a report from the U.S. Department of Commerce that problems may exist with a number of fish and shellfish sold in the state.

"Our concern is having a safe product," Schaefer said. "We want to be sure that our programs are effective and that we're doing everything we can to protect the health of the public." He added that he wanted to see how the problem could be addressed.

In other news:

La. oysters eaten in Md. tied to stomach complaint

WASHINGTON — Oysters taken from waters off Louisiana are suspected of causing a mysterious stomach ailment among consumers who did not eat them after the government said last night.

The government said that it would investigate the problem further and that it had no immediate plan to ban the oysters. It also said that it was investigating the possibility of a virus-like illness.

Food and Drug Administration officials said that more than 100 people were affected by a virus-like illness after eating the oysters.

In Louisiana, Mississippi and Maryland have reported getting the illness, the oysters. The oysters were caught in those areas of Louisiana were closed to harvesting Nov. 16.
Nucleotide Relatedness of SRSVs
104 bp within RNA Polymerase
Over a 3 day period, one sick fisherman contaminated 23,000 bushels of oysters sold in about 14 states with a single strain of norovirus.
Vomiting bug cases at record high

The virus has closed hospital wards.

The number of cases of the winter vomiting bug doubled over the last 12 months to reach their highest ever level, official figures have revealed.
Outbreaks aboard Cruise Ships, 2005

- 14 outbreaks on 10 ships
- Some repeated after cleanup
- By sequence, some linked to common sources
- Overcrowding –major risk
- Multiple modes of spread
  - Food, person-to-person
International Outbreaks of Norwalk like Virus
Enhanced Foodborne Outbreak Surveillance: CA, MD, TN; 2001-2002

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

Norovirus 52%

Unknown 22%

S. enteriditis 4%

C. perfringens 7%

S. aureus 15%

N = 27
Sporadic Norovirus Gastroenteritis in Adults: Emergency Department Patients

- 3 FoodNet sites (CT, NY, OR)
- 364 subjects enrolled
- 152 subjects with stool sample tested for all pathogens

Norovirus most common pathogen detected

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses</td>
<td>49 (32%)</td>
</tr>
<tr>
<td>Norovirus</td>
<td>30 (20%)</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>18 (12%)</td>
</tr>
<tr>
<td>Any bacteria</td>
<td>21 (14%)</td>
</tr>
<tr>
<td>Any parasite</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Any pathogen</td>
<td>73/152 (48%)</td>
</tr>
<tr>
<td>No pathogen</td>
<td>79/152 (52%)</td>
</tr>
</tbody>
</table>
The Global Challenge

Are noroviruses a problem in the developing world?
Etiology of Severe Diarrhea in Peruvian Children

- Bacteria: 20%
- Parasite: 5%
- Unknown: 10%
- Norovirus: 25%
- Rotavirus: 40%

Will this translate into mortality?

U. Parasher, 2003
Etiology of Diarrhea in Finnish Children

- Unknown: 39%
- Bacteria: 2%
- Parasite: 2%
- Rotavirus: 24%
- Norovirus: 19%
- Mixed/Other: 14%

X. Pang, 2000
Research Challenges

A Kapikian

B, C, D Prasad

Diagnostics
Receptors
Vaccines
Cell Culture
Animal models
Histoire Naturelle des Infections à Rotavirus

- La cause la plus fréquente de diarrhée sévère chez l’enfant
- Touchera tous les enfants avant l’âge de 5 ans
- Un virus “Democratic”
- La première infection est symptomatique
- Immunité naturelle est bonne
- Peu de souches circulantes
- L’amélioration de conditions d’hygiène n’influence pas l’infection
Distribution des 600,000 décès annuels dus au rotavirus

Parashar, 2005

1 dot = 1000 deaths
Importance de Rotavirus aux US

Risk

1 : 10^6
1 : 80
1 : 7
1 : 0.9

Events

20-40 Deaths
60-70,000 Hospitalizations
500,000 Outpatient visits
3.2 Million episodes

Cost: $400 M medical; >$1 B total
Diarrhea-associated hospitalizations by month & age among U.S. children < 5 years, 1979-1997

N=21,256

Rare or regionally common strains (23 strains total): P[4]G1 (1.3%), P[6]G2 (0.8%), P[6]G1 (0.6%), P[6]G8 (0.6%), P[4], G3 (0.5%)
The New York Times

F.D.A. Approves Vaccine for Childhood Diarrhea

By THE ASSOCIATED PRESS

Washington -- The Food and Drug Administration Monday approved the first vaccine against a leading cause of childhood diarrhea, a virus that hospitalizes 55,000 American children a year and kills one million in other countries.

Vaccine offers way to prevent child diarrhea

Rotavirus Vaccine Cuts Diarrhea Hospitalizations
Rhesus Rotavirus Vaccine

- Vaccins vivant oral
- 3 doses -- 2, 4, 6 mois
- Bien toléré
- Efficacité – >90% contre hospitalisations
- Efficacité évaluée au Venezuela
- Acceptabilité rapide aux USA malgré le prix ($38/dose)
# Calendrier Vaccinal aux USA --1999

<table>
<thead>
<tr>
<th>Age</th>
<th>Vaccine</th>
<th>Birth</th>
<th>1 mo</th>
<th>2 mos</th>
<th>4 mos</th>
<th>6 mos</th>
<th>12 mos</th>
<th>15 mos</th>
<th>18 mos</th>
<th>4-6 yrs</th>
<th>11-12 yrs</th>
<th>14-16 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td></td>
<td>Hep B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hep B</td>
</tr>
<tr>
<td>Diphtheria, Tetanus, Pertussis</td>
<td></td>
<td></td>
<td>Hep B</td>
<td></td>
<td></td>
<td>Hep B</td>
<td></td>
<td></td>
<td></td>
<td>DTaP</td>
<td></td>
<td>DTaP</td>
</tr>
<tr>
<td>H. influenzae type b</td>
<td></td>
<td></td>
<td></td>
<td>Hib</td>
<td>Hib</td>
<td></td>
<td>Hib</td>
<td></td>
<td>Hib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polio</td>
<td></td>
<td></td>
<td></td>
<td>IPV</td>
<td>IPV</td>
<td></td>
<td>Polio</td>
<td></td>
<td>Polio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td></td>
<td></td>
<td>Rotavirus</td>
<td></td>
<td></td>
<td>Rotavirus</td>
<td></td>
<td></td>
<td>MMR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles, Mumps, Rubella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MMR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Var</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Intussusception Among Recipients of Rotavirus Vaccine — United States, 1998–1999

On August 31, 1998, a tetravalent rhesus-based rotavirus vaccine (RotaShield®, Wyeth Laboratories, Inc., Marietta, Pennsylvania) (RRV-TV) was licensed in the United States for vaccination of infants. The Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrics, and the American Academy of Family Physicians have recommended routine use of RRV-TV for vaccination of healthy infants (1,2). During September 1, 1998–July 7, 1999, 15 cases of intussusception (a bowel obstruction in which one segment of bowel becomes enfolded within another segment) among infants who had received RRV-TV were reported to the Vaccine Adverse Event Reporting System (VAERS). This report summarizes the clinical and epidemiologic features of these cases and preliminary data from ongoing studies of intussusception and rotavirus vaccine.
Interval between Vaccine and Intussusception

Murphy TV, et al, 2001
Evaluation du risque d’invagination intestinale après RotaShield®

<table>
<thead>
<tr>
<th>Study</th>
<th>Author</th>
<th>Risk</th>
<th>Excess Cases for US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary Data</td>
<td>Livengood-NIP</td>
<td>1 in 2500</td>
<td>1600</td>
</tr>
<tr>
<td>Reassessment</td>
<td>Livengood-NIP</td>
<td>1 in 2500</td>
<td>888</td>
</tr>
<tr>
<td>Case Series</td>
<td>Murphy-NIP</td>
<td>1 in 4670</td>
<td>785</td>
</tr>
<tr>
<td>Case Control</td>
<td>Murphy-NIP</td>
<td>1 in 9474</td>
<td>361</td>
</tr>
<tr>
<td>Cohort Study</td>
<td>Kramarz-NIP</td>
<td>1 in 11,073</td>
<td>316</td>
</tr>
<tr>
<td>Ecologic Studies</td>
<td>Chang-NYS</td>
<td>&lt;1 in 17,000</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Simonsen-NIH</td>
<td>-11%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45-210 days</td>
<td>122</td>
</tr>
</tbody>
</table>
Taux d’incidence et nombre d’hospitalisations pour invagination intestinal par âge (mois)
VSD, 1991-1997

80% des les inv. int. chez les enfants âge de plus de 3 mois lors de la première dose
The next generation of rotavirus vaccines

Human rotavirus

G1P[8]

GSK Bio

Rotarix

Bovine rotavirus with single human rotavirus gene substitution

G1

P[8]

G2

G3

G4

Merck

RotaTeq
Human-Bovine Reassortant Rotavirus Vaccine - RotaTeq® (Merck)

- Pentavalent
- Liquid vaccine with buffer, stabilizer
- 3 doses, 2ml/dose,
- easy to administer
- Grows poorly - high dose ($10^7$-$10^8$), low shedding
## Clinical Trials of Rotateq

<table>
<thead>
<tr>
<th></th>
<th>Vac / Placebo</th>
<th>Outcome</th>
<th>Vac / Placebo</th>
<th>Efficacy (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S., Finland</td>
<td>2834/2839</td>
<td>Any</td>
<td>83 / 315</td>
<td>74 (67-80)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>1 / 51</td>
<td>98 (88-100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.S.</td>
<td>650 /650</td>
<td>Any</td>
<td>15 / 54</td>
<td>73 (51-86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mod/sev</td>
<td>10 / 42</td>
<td>76 (52-89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>0 / 6</td>
<td>100 (13-100)</td>
</tr>
</tbody>
</table>
GSK Attenuated Human Rotavirus Vaccine, Rotarix®

- **Monovalent**
- **Lyophilized vaccine, needs reconstitution**
- **2 doses, 1 ml/dose**
- **Grows well - low dose (10^{5.8}); high shedding (>50%)**
## Clinical Trials of Rotarix

<table>
<thead>
<tr>
<th></th>
<th>Vac/Placebo</th>
<th>Outcome</th>
<th>Vaccine Placebo</th>
<th>Efficacy (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finland,</td>
<td>245 / 123</td>
<td>Any</td>
<td>13 / 23</td>
<td><strong>72</strong> (42-87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>2 / 10</td>
<td><strong>85</strong> (42-97)</td>
</tr>
<tr>
<td>Brazil, Mexico,</td>
<td>464 / 454</td>
<td>Any</td>
<td>15 / 49</td>
<td><strong>70</strong> (46-84)</td>
</tr>
<tr>
<td>Venezuela</td>
<td></td>
<td>Severe.</td>
<td>5 / 3434</td>
<td><strong>86</strong> (63-96)</td>
</tr>
<tr>
<td>Latin America</td>
<td>10,159 / 10,010</td>
<td>Severe Hosp.</td>
<td>NA</td>
<td><strong>85</strong> (72-92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>85</strong> (77-94)</td>
</tr>
</tbody>
</table>
Efficacy of Rotarix

• 63,225 infants in 11 Latin American countries
• ~20,169 followed for severe GE until 12 mo.
• Efficacy - severe RVGE 85%
  G1 disease 92%
  non-G1 75%
  (G2 ~50%)

All GE hospitalizations 41%
# Safety from Intussusception

<table>
<thead>
<tr>
<th></th>
<th>Followup</th>
<th>Vac/Placebo</th>
<th>IS cases Vac/Pl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rotarix</strong></td>
<td>31 days</td>
<td>31,500/31,500</td>
<td>Dose 1 1 / 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose 2 5/5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total 6 / 7</td>
</tr>
<tr>
<td><strong>RotaTeq</strong></td>
<td>42 days</td>
<td>35,150/35,150</td>
<td>Dose 1 0 / 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose 2 4/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose 3 2/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total 6 / 5</td>
</tr>
</tbody>
</table>

Was Intussusception due to Rotashield alone?
Draft-- Recommendations for Pentavalent Bovine-Human Rotavirus Vaccine (PRV)

- Routine immunization of infants
- 3 doses at 2, 4, and 6 months of age
- Dose 1 between 6-12 weeks of age
- All doses by 32 weeks of age
- 4-10 week interval between doses
<table>
<thead>
<tr>
<th>WHO Region</th>
<th>Countries that have licensed Rotarix®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Americas</td>
<td>Argentina, Aruba, Bolivia, Brazil, Chile, Colombia, Costa Rica, Curaçao, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Jamaica, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Trinidad/Tobago, Venezuela</td>
</tr>
<tr>
<td>Africa</td>
<td>Burkina Faso, Cameroun, Central African Republic, Congo, DRCongo, Guinea, Ivory Coast, Kenya, Madagascar, Malawi, Mali, Mauritania, Mauritius, Nigeria, Senegal, South Africa, Togo</td>
</tr>
<tr>
<td>Europe</td>
<td>Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, UK</td>
</tr>
<tr>
<td>Middle East</td>
<td>Bahrain, Morocco, Oman, Pakistan, Qatar, Saudi Arabia, UAE, Yemen</td>
</tr>
<tr>
<td>Southeast Asia</td>
<td><strong>Bangladesh</strong>, Sri Lanka, Thailand</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>Australia, Hong Kong, Malaysia, New Zealand, Philippines, Singapore, Taiwan</td>
</tr>
</tbody>
</table>

From: Robin Bielik, PATH
## Status of Rotavirus vaccine licensure, as of 1 March 2007

**Merck RotaTeq™ licensure (47 countries):**

<table>
<thead>
<tr>
<th>WHO Region</th>
<th>Countries that have licensed RotaTeq®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Americas</td>
<td>Argentina, Canada, Curação, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Perú, Puerto Rico, USA</td>
</tr>
<tr>
<td>Africa</td>
<td>DR Congo, Guinea, Kenya, Niger, Rwanda, Togo</td>
</tr>
<tr>
<td>Europe</td>
<td>Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Turkey, UK</td>
</tr>
<tr>
<td>Middle East</td>
<td>0</td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>0</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>3 Australia, Hong Kong, Taiwan</td>
</tr>
</tbody>
</table>

From: Robin Bielik, PATH
Les vaccins vivant oraux -- seront ils efficaces dans les pays en développement ?

- **OPV**: Less immunogenic/more doses needed for children in India *(T. Jacob Johns)*

- **Cholera (Oralchol)**: Less immunogenic/higher titer needed in Thai/Indonesian studies *(Mike Levine)*

- **Oral RV**
  - **RIT**: Failed in Africa/Peru
  - **WC3**: Failed in Africa
  - **RRV**: Lower efficacy in Peru/Brazil
  - **GSK**: Being tested in S.Africa/Bangladesh
Timelines

The Rotavirus Vaccine Agenda
Reseau de rotavirus en Asie
2001-2003

China: 41%
Taiwan: 41%
Vietnam: 60%
Malaysia: 56%
Indonesia: 39%
Korea
Hong Kong: 29%
Myanmar: 56%
Reseaux regionaux de surveillance de rotavirus

Euro -2006

South America

South Asia

Africa

EMRO

Asia
Vaccins rotavirus - "le fruit le plus mur" des vaccins en développement

disease burden is large & global
principles to develop vaccines are well established
extensive past experience with clinical trials
achievable in 5-7 years
impact of vaccine should be measurable within one year

HIV
Tb
Malaria
Jap B
Dengue
MenC
Rotavirus
Celles qui font peur et celles qui tuent!

CJD
Anthrax
Smallpox
SARS/Ebola
monkeypox
West Nile Virus

ARI
NLV
diarrhea
Malaria/ flu
rotavirus
Papilloma/ Tbc
Hepatitis/ HIV

<1,000 Deaths/Year
>500,000

Media Attention
Public Health Importance

Deaths/Year

<1,000

>500,000
Utilisera-t-on le vaccin Rotavirus en Europe?

Quelles seront les facteurs clés de décision ?