Enteroviruses and Other Enteric Viruses

- Clinical Syndromes and Detection of Enterovirus Infection
- Pathogenesis of Hepatitis A Virus
- Viruses of Diarrhea
**Enteroviruses**

- >31 echoviruses
- >30 coxsackie A/B viruses
- >20 enteroviruses
- 3 polioviruses
- 1 Hepatitis A virus

**Picornaviridae**

**Rhinoviruses**

- >100 serotypes

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**Clinical Syndromes Associated with Enteroviruses**

- Aseptic meningitis
- Pericarditis, myocarditis
- Exanthems
- Muscle weakness/paralysis
- Conjunctivitis

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**Pathogenesis of Enterovirus Infection**

Transmission: fecal-oral route, respiratory route and direct contact
Hand, Foot and Mouth Disease

Numerous palmar lesion in an elderly woman. Note varying ages of lesions and cloudy fluid in many vesicles.

Fourteen month-old black child showing lesion on the hand and popular lesions on the lower arm.

Closeup view showing characteristic viral lesions of varying age. Youngest lesion is clear-fluid vesicle.

Detecting Enterovirus Infection

- Hand, foot and mouth disease is generally a clinical diagnosis!
- You could send fluid from vesicles to the lab to infect cells or do RT-PCR

Case: In late August, a 5 year old male is seen in the emergency room and exhibits fever, headache, photophobia, confusion and stiff/painful neck.
Outbreak of Aseptic Meningitis in Whiteside County, Illinois 1995

Classic cytopathic effect (CPE) of enterovirus

Polymerase Chain Reaction (PCR)
PCR Film Array technology for detection of viruses from clinical samples

YouTube:
The FilmArray – How It Works – NEW! (~5 min)

Inject Sample
Inject Hydration
Solution
Insert Pouch into Loading Station
Add Pouch to Film Array and Start Run

The FilmArray Pouch

Meningitis/Encephalitis (ME) Panel (FDA Cleared 10-8-15)

**Bacteria:**
- *E. coli K1*
- *H. influenzae*
- *L. monocytogenes*
- *S. agalactiae*
- *S. Pneumoniae*

**Viruses:**
- Cytomegalovirus (CMV)
- Epstein-Barr virus (EBV)
- Herpes simplex type 1 (HSV-1)
- Herpes simplex type 2 (HSV-2)
- Human herpesvirus 6 (HHV-6)
- Human Parechovirus
- Varicella zoster virus (VZV)

**Yeast:**
- Cryptococcus gattii/neoformans

* >95% of Community Acquired Causative Agents
* Sample Type: 200 µl Cerebrospinal Fluid (CSF)
What are the symptoms of EV-D68 infection?

- EV-D68 can cause mild to severe respiratory illness. Mild symptoms may include runny nose, sneezing, cough, and body and muscle aches. Severe symptoms may include wheezing and difficulty breathing. Children with asthma may have a higher risk for severe respiratory illness caused by EV-D68.

- How does the virus spread? Since EV-D68 causes respiratory illness, the virus can be found in an infected person’s respiratory secretions, such as saliva, nasal mucus, or sputum. EV-D68 likely spreads from person to person when an infected person coughs, sneezes, or touches a surface that is then touched by others.

- What time of the year are people most likely to get infected? In the United States, people are more likely to get infected with enteroviruses in the summer and fall.

More info at www.cdc.gov/ev-d68

Antiviral Therapy: Pleconaril

- Developed from our knowledge of virus replication and assembly of capsid proteins

- The drug incorporates into virus particles, changes the shape of the particle, altered particle can not bind receptor and infect a new cell

Enterovirus particle
Pleconaril

First anti-viral drug with activity against picornaviruses, the predominant cause of the common cold.
Antiviral Therapy

- Pleconaril: the greasy sausage blocker
- Incorporates into virus particles, changes the shape of the particle, altered particle can not bind receptor and infect a new cell

Antiviral Therapy for Enteroviruses

- Pleconaril: the greasy sausage blocker
- When to use it?
  - Not for routine infections (FMD)
  - Possible for disseminated infections in immunocompromised patients with Enterovirus D68?

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Oral acquisition

Crosses Intestine

Blood

Bile

Stool

Typical Course of Acute Hepatitis A Virus (HAV) Infection

Clinical Illness

IgG anti-HAV

IgM anti-HAV

ALT

Weeks after Exposure

Response (Relative Titer)

Injection of virus

Weeks after Exposure

HAV in stool

HAV in blood

HAV in blood
Number of hepatitis A cases, by date of eating at Restaurant A and illness onset – Monaca, Pennsylvania, 2003

<table>
<thead>
<tr>
<th>Dining dates</th>
<th>Onset dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>October Day and month</td>
<td>November Day and month</td>
</tr>
<tr>
<td>September Day and month</td>
<td>October Day and month</td>
</tr>
</tbody>
</table>

IgM Capture Assay

- Substrate
- Anti-Mouse Ig
- Hepatitis A Viral Antigen
- Anti-IgM Capture Antibody
- Plastic Solid Phase
- Mouse Anti-HAV
- Patient’s Serum (IgM anti-HAV?)

Hepatitis A Virus Vaccine

- Killed virus vaccine (IM injection)
- Effective in preventing disease
- Effective treatment post-exposure!!
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**Impact of Gastroenteritis (24 hrs)**

- 200 million people on earth have gastroenteritis
- Amount of diarrheal water = amount water over Victoria Falls in 1 minute

**Viruses of Diarrhea**

- Rotavirus
- Caliciviruses: Noroviruses
- Astroviruses
- Adenoviruses
- Coronaviruses
Rotavirus

Disease
- Epidemic diarrhea of young children

Transmission
- Human contact

Symptoms
- Dehydration
- Diarrhea
- Vomiting
- Fever

Estimated number of annual deaths, hospitalizations, emergency department visits, and episodes of rotavirus gastroenteritis among children aged <5 years – United States

- 20-60 deaths
- 55,000-70,000 hospitalizations
- 205,000-272,000 Emergency department visits and 410,000 outpatient/office visits
- 2.7 million episodes

Rotavirus particles visualized by immune electron microscopy in stool filtrate from child with acute gastroenteritis. 70-nm particles possess distinctive double-shelled outer capsid.
Detection of rotavirus by ELISA/EIA

- OR detect viral RNA using the film array PCR technique on fecal samples

Impact of Rotavirus Diarrheal Disease: US

- Annually
  - 3.1 million cases
  - 65-75,000 hospitalizations
  - 125 deaths
  - $1 billion cost

- Map showing the distribution of rotavirus across the US from November to April.
Rotavirus Pathogenesis

- Infection primarily restricted to villus epithelium of the small intestine
- Outcome of infection is age-related
  Symptomatic infection occurs in young children 6 months to 2 years of age
  (also in mice up to 2 weeks of age)

Mechanism of Rotavirus Diarrhea

- Previous dogma
  - Malabsorption secondary to destruction of enterocytes results in osmotic diarrhea.
- New idea
  - Rotavirus encodes a viral enterotoxin.
  - The viral enterotoxin (NSP4) causes the diarrhea.
Properties of Rotavirus
Enterotoxin NSP4

- Functions in viral morphogenesis: mediates acquisition of a transient membrane envelope as subviral particles bud into the endoplasmic reticulum (ER).
- Mobilizes calcium: releases Ca\textsuperscript{2+} from internal stores
- Causes diarrhea when injected into animals

![Graph showing percent with antibody across different age groups (6-12, 13-24, 25-36, 37-48 months)].

Each year, rotavirus threatens 3.5 million children. Now......
there’s prevention.

New Rotavirus vaccine
Approved Feb, 2006!!

Rotateq – a pentavalent,
Live-attenuated vaccine

RotaTeq Vaccine

- Live-attenuated, pentavalent vaccine
- Efficacy:
  - 98% protection against severe disease
  - 86% reduction in clinical visits
Efficacy of RotaTeq® against rotavirus gastroenteritis by disease severity

<table>
<thead>
<tr>
<th>Disease severity</th>
<th>No of cases</th>
<th>% Efficacy</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine (N=3,484)</td>
<td>97</td>
<td>369</td>
<td>73.8</td>
</tr>
<tr>
<td>Placebo (N=3,499)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>97</td>
<td>369</td>
<td>73.8</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td>57</td>
<td>98.2</td>
</tr>
</tbody>
</table>

Efficacy of RotaTeq® in reducing the rate of healthcare use for rotavirus gastroenteritis

<table>
<thead>
<tr>
<th>Type of contact</th>
<th>No of cases</th>
<th>% Rate reduction</th>
<th>% Rate reduction (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td>6/34,035</td>
<td>144/34,003</td>
<td>95.8</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalizations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency department visits</td>
<td>14/34,035</td>
<td>225/34,003</td>
<td>93.7</td>
</tr>
<tr>
<td>Office visits</td>
<td>13/2,834</td>
<td>98/2,839</td>
<td>86.0</td>
</tr>
</tbody>
</table>

Generating a Rotavirus Vaccine

- Rotavirus has 11 segments of double strand RNA
- During viral replication and packaging, the viral segments can re assort
Rotavirus is a dsRNA virus with 11 segments
- There are 5 major types of rotavirus
- Antibodies to VP4 and VP7 are neutralizing

Important concept: Viruses with segmented RNA genomes can undergo **REASSORTMENT**

Dual infection of enterocyte by 2 parent viruses

Progeny viruses

The concept of reassortment is important for:

1. Generating vaccines for rotavirus:
   - Start with an attenuated virus (human or bovine)
   - Reassort RNA segments to generate vaccine strains that express different VP7s
   - Combine the vaccine strains to make a pentavalent vaccine that elicits neutralizing antibody to many different genotypes of rotavirus

2. Antigenic **SHIFT** of influenza virus (next lecture!!)
Sample exam questions:

Reassortment occurs in which of these viruses:

A) Polioviruses and Enteroviruses  
B) Rotavirus and Influenza virus  
C) Yellow fever virus and Dengue virus  
D) Ebola virus and Marburg virus  
E) SARS coronavirus and MERS coronavirus

The answer to this question is B because both rotavirus and influenza viruses have segmented RNA genomes that can undergo reassortment if multiple viruses infect the same cell.

Prevalent strains of rotavirus – United States, 1996-2005

- P[8]G1 (78%)
- P[4]G2 (9%)
- P[8]G4 (1%)
- P[6]G9 (2%)
- P[8]G3 (2%)
- P[8]G9 (4%)
- Other (4%)
For 2007-2009:
64,855 fewer hospitalizations saves $278 million in treatment costs

Summary for Rotavirus

• Common cause of childhood diarrhea
• Transmission by fecal-oral contamination
• Pathogenesis due to viral enterotoxin nsp4
• Effective live-attenuated vaccines protect from severe disease
Number of passengers and crew members reporting to the ship’s infirmary with symptoms of acute gastroenteritis during two consecutive 7-day cruises on cruise ship A, by date of illness onset – Vancouver to Alaska, July 18-August 1, 2002

Characteristics of Noroviruses

- (+) RNA virus with capsid
- Resistant to environmental pressures: drying, detergents, acids and temperature
- Transmitted by fecal-oral route
- Causes outbreaks of gastroenteritis
- Disease resolves after 48 hours - generally without serious consequences in healthy adults

Noroviruses

- No antivirals currently available
- No vaccines currently available….
- Multiple genotypes exist, so repeated experiences of similar disease is possible.
- Detection is by RT-PCR of fecal sample
Noroviruses

• About 20% of people do not express the receptor for Group 1 noroviruses
• The viral surface antigens change over time, making vaccine development challenging. Colors show amino acid changes over time.

Original Article
Norovirus Vaccine against Experimental Human Norwalk Virus Illness

VLP: virus-like particle vaccine


Odds Ratios for Viral Gastroenteritis and Norwalk Virus Infection, According to Prechallenge Histo-Blood Group Antigen–Blocking Antibody Titer and Analysis.

Table 1. Odds Ratios for Viral Gastroenteritis and Norwalk Virus Infection, According to Prechallenge Histo-Blood Group Antigen–Blocking Antibody Titer and Analysis.∗

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Prechallenge Histo-Blood Group Antigen–Blocking Antibody Titer</th>
<th>Postchallenge Histo-Blood Group Antigen–Blocking Antibody Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-HBGA Blocking Serum Titer (IU/mL)</td>
<td>Anti-HBGA Blocking Serum Titer (IU/mL)</td>
</tr>
<tr>
<td></td>
<td>No Neutralization (%)</td>
<td>Yes Neutralization (%)</td>
</tr>
<tr>
<td>Viremia</td>
<td>30.0 (50)</td>
<td>31.0 (50)</td>
</tr>
<tr>
<td>Norovirus</td>
<td>3.0 (60)</td>
<td>3.0 (60)</td>
</tr>
</tbody>
</table>

Conclusions
This norovirus VLP vaccine provides protection against illness and infection after challenge with a homologous virus.
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  - Rotavirus
  - Noroviruses

Seven Question Approach
Test your knowledge by taking the 7 question quiz:

Loyola wired
LUMEN
MHD
Educational resources
Virus 7 question approach quizzes
Pick your favorite!