**VIRUSES & PRIONS**
TRANSMITTED VIA FOOD AND WATER
Dean O. Cliver

**Food- and waterborne viruses**
- Human enteric viruses
  - Specific for humans
  - Infect perorally, shed in feces
- Classification: size, nucleic acid type, appearance
- Replication: inside a host cell

**Enteric virus groups**

<table>
<thead>
<tr>
<th>Size, nm</th>
<th>NA strands</th>
<th>RNA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>25–35</td>
<td>single</td>
<td>astro-calici-picorna-</td>
<td>parvo-</td>
</tr>
<tr>
<td>70–85</td>
<td>double</td>
<td>reo-</td>
<td>adeno-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rota-</td>
</tr>
</tbody>
</table>

**Causes of foodborne outbreaks, U.S., ’98–’02**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Agent</th>
<th>Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Norovirus</td>
<td>27,121</td>
<td>21.2</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella</td>
<td>16,821</td>
<td>13.1</td>
</tr>
<tr>
<td>3</td>
<td>C. perfringens</td>
<td>6,274</td>
<td>5.2</td>
</tr>
<tr>
<td>4</td>
<td>Escherichia coli</td>
<td>4,864</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>Shigella</td>
<td>3,677</td>
<td>2.9</td>
</tr>
</tbody>
</table>

**E.I.D. estimates, 1999:**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Ann. incidence</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noroviruses</td>
<td>9,200,000</td>
<td>66.6</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>981</td>
<td>0.8</td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>613</td>
<td>0.5</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>571</td>
<td>0.4</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>4,170</td>
<td>&lt;0.1</td>
</tr>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>Staph. aureus</td>
<td>2,766</td>
<td>2.2</td>
</tr>
<tr>
<td>7</td>
<td>Campylobacter</td>
<td>1,440</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>Hepatitis A virus</td>
<td>981</td>
<td>0.8</td>
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<td>571</td>
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</table>
Icosahedral symmetry

RNA virus replication:
- Virus
- Cytoplasm
- Cell membrane
- Nucleus

RNA virus replication (2):
- Cell membrane
- Cytoplasm
- Nucleus

RNA virus replication (3):
- Cell membrane
- Cytoplasm
- Nucleus

RNA virus replication (4):
- Cell membrane
- Cytoplasm
- Nucleus
- Viral RNA

RNA virus replication (5):
- Cell membrane
- Cytoplasm
- Nucleus
- Viral peptide
RNA virus replication (6): coat proteins
- strand RNA

RNA virus replication (7): coat proteins

RNA virus replication (8): progeny virus

RNA virus replication (9): progeny virus

RNA virus replication (10): lysis

Norovirus EPA
Noroviruses

- CDC
  - est. 9.2 million foodborne/yr
- CAST: 181,000 cases/year, 0 deaths, $890

History and naming

- Norwalk, Ohio, gastroenteritis outbreak, 1972
- Small round structured viruses (SRSV), “Norwalk-like”
- Calicivirus group — small (~30 nm?), single-stranded RNA, protein coat has “dimples”

The disease

- Virus from ill or convalescent person, via feces or vomitus
- Colonization of intestines — incubation: 1–2 days

The disease 92)

- Severe diarrhea & vomiting — 12–60 hr (usually 24–48 hr), virus shedding up to 7 days
- Antibody is not protective

Transmission/Control

- Routes — person-to-person, or
  - via water
  - “undercooked” shellfish (cf. HA)
  - food handled by an infected person
- Prevention — sanitation, cooking

Diagnosis of noroviral gastroenteritis

- Clinical: vomiting & diarrhea, ≥18 hr incubation
- Virus in feces
  - Tests for particles or antigen
  - Tests for viral genome
- Antibody production (serogroups)
**Hepatitis A virus**

- Picornavirus:
  - ca. 28 nm diameter
  - single (+) strand RNA
  - coat protein comprises 60 copies of each of four structural polypeptides
- Relatively resistant to heat and to drying

**Estimated annual hepatitis A**

- CDC: ~11,000 cases
- CAST:
  - 4,800–35,000 cases
  - ≤14 deaths
  - $5030/case

**History**

- Viral hepatitis recognized ca. time of World War II
- Fecal-oral transmission of “infectious hepatitis” (now hepatitis A) recognized much later

**History (2)**

- 5–6 hepatitis viruses now known
- Only hepatitis A is known to be transmitted via food and water in North America

**The disease**

- Virus in feces of infected person
- Entry via intestines
- Liver colonized
  - Infected cells destroyed by host’s immune response
  - Incubation 15–50 days (average 28–30)
**The disease (2)**
- Virus shed in feces 1–2 weeks before onset
- Illness: fever, malaise, anorexia, nausea, abdominal discomfort—jaundice (?)
- Usually complete recovery after a few weeks, permanent immunity

**Transmission/Control**
- Routes — person-to-person, or via water (drinking, irrigation?), “undercooked” shellfish, or food handled by an infected person
- Prevention — sanitation, cooking, **vaccination** (U.S., 1995)
- Food as a vehicle — 5%?

**Events In Hepatitis A Virus Infection**

**Other gastroenteritis viruses**
- Astroviruses — occasionally foodborne, some replicate in cell culture
- Rotaviruses — more often infant diarrhea than foodborne disease
- Adenoviruses — serotypes 40 & 41, not known to be foodborne
- Coronavirus — questionable cause of human diarrhea, foodborne once?

**Astroviruses**

**Rotaviruses**
Other viruses and food
- Human enteroviruses (polioviruses, coxsackieviruses, echoviruses) — rare in U.S.
- Hepatitis E virus — water, food?
- Tick-borne encephalitis virus — milk & milk products, Slovakia
- “Non-problems” — hepatitis B, C, & D; herpes, HIV, hantavirus

Detection & monitoring:
- Diagnosis, adapted
- Cell culture
  - Cytopathic effects
  - Plaques
Detection of viruses in food

- Sample processing
  - liquefaction
  - clarification
  - concentration
- Test methods
  - probes
  - RT-PCR
  - antigen capture

RT-PCR of hepatitis A

“Indicators”

- Bacteria — fecal coliforms, *Escherichia coli*, etc.
- Viruses — vaccine polioviruses
- Phages — fecal origin, resemble human viruses (?)

“Male-specific” coliphages

Preventing transmission

Food vehicle
Water vehicle

Shellfish as vehicles

Filter feeders

Digestive tract eaten

Raw, or slightly cooked

Protection from heat
Other food vehicles

Prevention
- Sanitation (handwashing)
- Depuration of shellfish
- Cooking & other means of inactivation

Viruses are inert — can’t multiply in food

Persist, or lose infectivity (“inactivation”)

Cooking inactivates viruses

Freezing preserves viruses
UV inactivates viruses on surfaces or in water

Some viruses are inactivated by drying

Chlorine inactivates viruses on surfaces or in water

Now for something completely different:

PRIONS!

Transmissible spongiform encephalopathies (TSEs)

- Accumulation of abnormal prions in brain leads to spongiform degeneration
- All are fatal
- Some are “contagious”

Prions are

- Low MW peptides found in CNS & some other organs.
- Normal folding depends on amino acid sequence.

\[
\text{normal prion (PrP}^C\text{)} \quad \bigcirc \quad \text{abnormal prion (PrP}^\text{Sc}\text{)} \quad \triangle
\]
Normal human prion protein

Abnormal prion folding

PrPSc in neuron

Normal human prion folding

Normal PrP cycle

PrPSc in neuron

Extracellular PrPSc
### “Old” TSEs
- Scrapie in sheep
- Creutzfeldt-Jakob disease (CJD), sporadic, etc., in humans,
- Transmissible mink encephalopathy
- Chronic wasting disease (deer, elk)

### “New” TSEs
- Bovine spongiform encephalopathy (BSE) — “mad cow disease”
- Feline spongiform encephalopathy
- New variant CJD (vCJD) in humans

### Prion “infection”

* (bovine) abnormal
* normal (bovine)

![BSE brain](image)

### BSE in cattle, UK
- April 1985 to December 2004, 184,131 confirmed cases of BSE (3–5-yr incubation)
- Control by not feeding rendered bovine meat-and-bone meal (MBM) to cattle — slow enforcement

### BSE in cattle, UK, 2
- Slaughter of affected herds
- Enormous research effort
- No BSE prions found in red meat (voluntary muscle) or milk
- Vertical transmission “unlikely”
- Carcass disposal precautions
**BSE in cattle elsewhere**
- Some cattle, much beef, and a lot of MBM exported from UK to other countries
- Now ca. 30 countries have BSE (few thousand cases), all in Europe except Japan, Israel, Canada, & US, so far.

**Mad cow?**

**Non-transmission by prion**

- (sheep scrapie) abnormal → contact → no change → normal (human)

**Inter-species transmission**
- Ca. 1994, TSE in cats (UK), including zoo species
- In 1995, ~CJD in young people, UK — “vCJD” (>10-yr incubation?)
- vCJD differs in more than age distribution of victims

**Prion “cross-infection”**

- (bovine) abnormal → contact → re-configuration → normal (human)

**Non-transmission by prion**

- (bovine) abnormal → contact → no change → normal (canine)
Impact of vCJD

• ~165 people in UK, 37 in the rest of the world affected by 04/07
• Even in UK, <CJD rate (28 vs 48 in peak year, 2000; 5 vs 57, 2006)
• Far less than deaths from other foodborne diseases
• HUGE reaction

Impact of vCJD, 2

• Specified bovine offals banned, most BSE countries
• Cattle >30 months old not eaten in UK, carcasses incinerated (no longer)
• Slaughter cattle >30 (24?) months old tested, other BSE countries

Impact of vCJD, 3

• Genetic susceptibility — all “primary” vCJD patients tested have been homozygous for methionine at codon 129 of their prion gene (40% of population)
• Restrictions on blood donation and use (3 probable UK cases)

US measures

• No mammalian MBM can be fed to food-source ruminants
• Restrictions on blood donation
• Scrutiny of biologicals
• Slaughter of “downer” cattle prohibited

US measures, 2

• No “risk materials” in human food supply from animals >30 months old
• Other prohibitions pending
• More testing of “downers,” dead-on-farm, suspects at slaughter
Dorsal root ganglion

Normal human brain, H&E

sCJD, H&E stain

vCJD, H&E stain

Normal human brain, IHC

sCJD, IHC
**Drama in North America — chronic wasting disease**
- Deer & elk, Colorado, Wyoming
- Other states, Canadian provinces
- Environmental transmission (feces?)
- Transmissible to humans??
- Processing carcasses — food safety?
- Now “upstaged” by BSE

**Summary**
- Human enteric viruses, fecal contamination
- Cooking or other means of inactivation (depuration)
- Detection vs. indicator systems for monitoring

**Summary, 2**
- Prion diseases are here in North America.
- Threat to human health is minimal.
- Measures being imposed may well lessen overall food safety.