Hepatitis A Outbreaks In Australia
Molecular Epidemiology

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Hepatitis A Virus

Transmission:
Faecal-oral route – contaminated food & water

Disease:
Jaundice, dark urine - severity of symptoms increase with age at infection; associated with acute infection only

Prevalence:
Endemic in Africa, Asia, Latin America; low prevalence in Australia, New Zealand, North America & most European Countries.
Hepatitis A Seroprevalence

Anti-HAV Prevalence

- High
- High/Intermed.
- Intermediate
- Low
- Very Low
HAV Morphology
Virus Characteristics

• Member of the *Picornaviridae*

• Non-enveloped, 27-32 nm in diameter

• Plus-sense single-stranded RNA genome, 7.5 kb

• Six genotypes (I – VI; originally VII) divided into subtypes
  - Human strains mostly genotype IA & IB, IIIA & IIIB

• Only a single serotype

• *No clinical significance associated with genotype*
Hepatitis A Virus

- Two distinct hepatitis agents shown by Krugman (1967)
- HAV particles shown by EM by Feinstone (1973), confirmed by Locarnini (1974)

**Stability**
- infectious for > 1 month
- stable at 60°C for several hours
- stable at low pH
- inactivated by chlorine – sodium hypochlorite 3-10 mg/L for 5-15 min; 10-15 ppm residual chlorine for 30 min
Diagnostic Markers of Hepatitis A

FIG. 1. Virologic, immunologic and biochemical events during the course of experimental hepatitis A virus infection in chimpanzees inoculated intravenously with human HAV, strain HLDZ. ALT, alanine aminotransferase. (Adapted from reference 157 with permission of the publisher.)

Recent Multijurisdictional Foodborne Hepatitis A Outbreaks in Australia

• Wallis Lake (1997) - contaminated oysters
  - approx 500 cases

• Semi-dried tomatoes (2009) – > 300 cases

• Mixed berry outbreak (2015) – < 40 cases
Diagnostic Assays (VIDRL)

• DiaSorin anti-HAV IgM + total antibody

• HAV RNA *RealStar* Real-Time PCR assay

• In-house nested HAV RT-PCR for genotyping
  – Use three different primer sets
  – Sequence
  – BLAST, CLUSTAL and phylogenetics as required
FIG. 2. Schematic representation of the HAV genome organization, translation products, and regions used for amplification. The area encoding the polyprotein is represented by solid box and the proposed cleavage lines by vertical lines. Regions commonly used for PCR amplifications are as follows: region 1, C terminus of VP3 region (nt 2,000 to nt 2,226); region 2, N terminus of VP1 region (nt 2,172 to nt 2,415); region 3, VP1/VP2A junction region (nt 2,084 to nt 2,217); region 4, VP1-VP2B region (nt 2,896 to nt 3,289); region 5, entire VP1 region (nt 2,172 to nt 3,125); and region 6, VP3-VP2B region (nt 2,133 to nt 3,289). Nucleotide position numbering is according to the HMI75 sequence (46).
Wallis Lake (1997)

- Large estuarine lake – Sydney Rock Oysters
- Association between oysters and hepatitis A established by matched case-control study
- HAV PCR used to detect virus in oysters
- Several potential sources of environmental contamination identified

Conaty et al Epidemiol Infect 2000
Semi-dried Tomato HAV Outbreak (2009)

- 230/259 serum samples anti-HAV IgM positive
- 182 samples HAV RNA positive by VP1/P2A primers

Donan et al. Clin Inf Dis 2009
Analysis of sequences shows 152/182 were identical, and subtype IB.
Putative Source – Semi-dried Tomatoes

- Samples of semi-dried tomatoes sent to French laboratory with accreditation for food testing
- Of 63 samples, 22 had detectable HAV RNA, 11 were quantifiable, only one sequenced
- Region sequenced was in N-terminal VP1 (VIDRL used C-terminal VP1/P2A junction)
- This sequence only 140 nucleotides, insufficient for accurate genotype analysis

Donan et al. Clin Inf Dis 2009
Molecular Link – Source and Recipients

- VIDRL – designed PCR primers to VP1 region
- Amplified a subset of patient samples (HAV IB by VP1/P2A)
- Sequenced and compared to limited French sequence data
Sequences from putative source and patient samples show 100% identity.
Frozen Berries HAV Outbreak - 2015

- Early January 2015 – Case 1
- Case 2 and 3 – Feb 1 and Feb 6, 2015
- Feb 12 – DHHS confirms possible link to frozen mixed berries > OzFoodNet Feb 13
- Feb 14 – Company’s frozen mixed berries recalled

Q. What do you do if you have eaten frozen berries?

A. Panic. See the doctor and get tested
Beginning of the Onslaught

- Samples for testing anti-HAV Total & IgM
- Samples HAV PCR testing
- Case Definitions by DHHS
- GP notification
- Contacted local Pathology labs to only forward anti-HAV IgM positive samples
Initial Molecular Investigation

- HAV RNA detected by RealStar assay
- HAV RNA amplified by VP1/P2A PCR primers
- Sequence analysis revealed HAV genotype IA
Frozen Berry Investigation

- 35 confirmed cases
- 28 had a risk factor of eating mixed berries
  - 3 identified as secondary cases; 2 could not recall brand of berries; two unknown risk factors
- VIDRL identified 19 cases infected with HAV genotype IA – sequences identical in VP1/P2A
- All 35 confirmed cases had HAV IA with identical sequence (16 tested in QLD)
- Sequenced 31 other HAV isolates unrelated to the outbreak - two unrelated clusters identified 2 x IA (WA); 3 x IB (NSW)
HAV in Berries

• One confirmed case had remaining berries in their freezer

• Sent by DHHS (VIC) to the South Australian Research and Development Institute (SARDI)

• HAV RNA isolated and sent to VIDRL

• Titre estimated to be 38-584 HAV copies/gm
HAV Berry v Prototype HAV
Outbreak Sequence

100% Identity
HAV Berry Sequence v Unrelated HAV IA

Formatted Alignments
Phylogenetic Tree of HAV Isolates
Source of Contamination?

- Water used for irrigation
- Food handler harvesting berries
- Food handler packing berries
Contributing Factors

• HAV more difficult to inactivate on produce with rougher surfaces (strawberries, blackberries)

• Unlikely that raspberries underwent any sanitization due to produce fragility

• Little reduction in titre of HAV with freezing (<1 \log_{10} after 90 days)
Local BLAST Database of HAV Sequences

• Established using software program “Geneious”

• Database design to contain:
  – HAV sequences from GenBank
  – Representative sequences of past HAV outbreaks
  – HAV sequences from sporadic HAV RNA positive samples

• BLAST new query sequences against this database
Proposed HAV Genotyping Protocol

• Retrospective genotyping using HAVNET primers
  – HAVNET is a global network of reference laboratories, focused on HAV
  – Map worldwide distribution of HAV strains by sharing sequences from common PCR primers

• Evaluate Next Gen Sequencing (HAV full genome)

• HAV isolation from food sources (MDU – PHU)
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