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



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



Nainan et al (2006). Clin Micro Reviews 19:63

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Recent Multijurisdictional Foodborne Hepatitis A Outbreaks in Australia

- Wallis Lake (1997) contaminated oysters
 - 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

HAV Genome Organization





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Wallis Lake (1997)

- Large estaurine 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

Semi-dried Tomato HAV Outbreak (2009)

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





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

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



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 sites by vertical lines. Regions commonly used for PCR amplifications are as follows: region 1, C terminus of VP3 region (nt 2,020 to nt 2,226); region 2, N terminus of VP1 region (nt 2,172 to nt 2,415); region 3, VP1/P2A junction region (nt 2,984 to nt 3,217); region 4, VP1-P2B region 1, C 1,272 to nt 2,217); region and region (nt 2,395 to nt 3,289); Nucleotide position numbering is according to the HM175 sequence (46).

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Sequences from putative source and patient samples show 100% identity

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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) 20

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

Formatted Alignments



HAV Berry Sequence v Unrelated HAV IA

Formatted Alignments

	10	20	30	40	50	60
3230-15513393 RIAL-VP1-N{R&C}P	TTATCTTTCA	GCAATCAAT	T G G A A T G C T T T O	GCTCCTCTTTATC	ATGCTATGGATG	TTAC
3422-15524999 FS15-0316A-VP{PN}	ТТАТСТТТСА	A G C A A T <mark>C</mark> A A T T	T G G A A T G C T T T G	GCTCCTCTTTATC	ATGCTATGGATG	TTAC
3389-15527438 ADPA-VP1-N{R&C}P	ТТАТСТТТСА	A G C A A T <mark>T</mark> A A T T	T G G A A T G T T T T G	GCTCCCCTTTATC	ATGCTATGGATG	TTAC
3405-15529162 SIWI-VP1-N{R&C}P	ТТАТСТТТСА	A G C A A T <mark>T</mark> A A T T	T G G A A T G T T T T G	GCTCCTTTTTATC	ATGCTATGGATG	TTAC
	ΤΤΑΤΟΤΤΤΟΑ	GCAATYAATT	TGGAATGYTTT	<u>GCTCCT</u> C <u>TTTATC</u>	ATGCTATGGATG	TTAC
	70	80	90	100	110	120
3230-15513393 RIAL-VPI-N{R&C}P	TACACAGGTT	G G A G A T G A T T	CAGGAGGTTTT	F C A A C A A C A G T T T	C T A C A G A G C A G A	ATGT
3422-15524999 F\$15-0316A-VP{PN}	TACACAGGTT	G G A G A T G A T T	CAGGAGGTTTT	F C A A C A A C A G T T T	C T A C A G A G C A G A	ATGT
3389-15527438 ADPA-VP1-N{R&C}P	CACACAGGTT	G G A G A T G A T T	CAGGAGGTTTT	Г С А А С А А С А G T T T	C T A C A G A A C A G A	ATGT
3405-15529162 SIWI-VP1-N{R&C}P	TACACAAGTT	GGAGATGAT	CAGGAGGTTTT	F C A A C A A C A G T T T	CCACAGAACAGA	ATGT
	ТАСАСАББТТ	T G G A G A T G A T T	CAGGAGGTTTT	TCAACAACAGTTT	CTACAGARCAGA	АТСТ
	130	140	150	160	170	180
3230-15513393 RIAL-VP1-N/R&C}P	TCCTGATCCC	CAAGTTGGCA	TAACAACCATC	AGGGACTTAAAAG	GGAAAGCCAATA	<u> </u>
3422-15524999 ES15-0316A-VP{PN}	TCCTGATCCC	CAAGTTGGCA		AGGGACTTAAAAG	G G A A A G C C A A T A	6666
3389-15527438 ADPA-VP1-N{R&C}P		CAAGTTGGCA	TAACAACCATG	AGGGACTTAAAAG	G G A A A G C C A A C A	6666
3405-15529162 SIWI-VP1-N{R&C}P		CAAGTCGGTA	TAACAACCATG	AGGGACTTAAAAG	GAAAAGCCAATA	6666
	TCCTGATCCC	$C \land \land G T T G G C \land$	TAACAACCATG	AGGGACTTAAAAG	G G A A A G C C A A T A	0000
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	190	200	210	220	230	240
3230-15513393 RIAL-VP1-N{R&C}P	AAAGATGGAT	GTTTCAGGAG	TGCAAGCACCC	GTGGGAGCTATTA	CAACAATTGAGG	ATCC
3422-15524999 FS15-0316A-VP{PN}	AAAGATGGAT	T G T T T C A G G A G	TGCAAGCACCC	GTGGGAGCTATTA	CAACAATTGAGG	ATCC
3389-15527438 ADPA-VP1-N{R&C}P	AAAGATGGAT	T G T T T C A G G A G	TGCAAGCACCT	GTGGGAGCTATTA	CAACAATTGAGG	ATCC
3405-15529162 SIWI-VP1-N{R&C}P	AAAGATGGAT	T G T T T C A G G A G	TGCAAGCACCT	G T G G G A G C T A T <mark>C</mark> A	CAACAATTGAGG	ATCC
	AAAGATGGAT	F G T T T C A G G A G	TGCAAGCACCY	GTGGGAGCTATTA	CAACAATTGAGG	АТСС
	250	260	270	280	290	300
3230-15513393 RIAL-VP1-N{R&C}P	AGTTTTAGCA	A A G A A A G T G C	CTGAGACATTT	C C T G A A T T G A A G C	CTGGAGAATCCA	GACA
3422-15524999 FS15-0316A-VP{PN}	AGTTTTAGCA	A A G A A A G T G C	CTGAGACATTT	CCTGAATTGAAGC	CTGGAGAATCCA	GACA
3389-15527438 ADPA-VP1-N{R&C}P	AGTTTTAGCA	A A G A A A G T G C	CTGAGACATTT	C C T G A A T T G A A A C	CTGGAGAATCCA	GACA
3405-15529162 SIWI-VP1-N{R&C}P	AGTTTTAGCA	AAGAAAGTGC	CTGAGACATTT	C C T G A A T T G A A G C	CTGGAGAATCCA	GACA
	AGTTTTAGCA	AAGAAAGTGC	CTGAGACATTTO	C C T G A A T T G A A G C	CTGGAGAATCCA	GACA
	310	320	330	340	350	360
3230-15513393 RIAL-VP1-N{R&C}P	TACATCA	020	000	040	000	000
3422-15524999 ES15-03164-VP/PNI	TACATCA					
3389-15527438 ADPA-V/P1-N/P8-C1D	TACATCA					
3405-15529162 SIWI-VP1-NIDR.CLD	TACATCA					
	TACATCA					



0.01

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 log₁₀ 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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- Food Safety Unit and investigating officers, DHHS
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