DETECTION OF HEPATITIS A VIRUS RNA IN SLICED HAM

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Introduction - HAV

- Picornaviridae, Hepatovirus;
- Non-enveloped, single + stranded RNA virus;
- Icosahedral capsid, around 28 nm in diameter;
- 3’ – polyadenylated;
- At the 5’ end of the RNA strand is a viral protein called VPg;
- Replication lifecycle in 8 hrs, host cell zero protein output in 30 min ;
- There is only one serotype of HAV, but multiple genotypes (I-III infect humans);
Introduction – Infection route

- Causes acute liver infection with a discrete onset of symptoms (e.g., fever, malaise, and nausea), followed in several days by jaundice.
- Carried in the stool of infected people;
- Spread when an infected person does not wash their hands after using the bathroom and then touches food, a surface, or another person's mouth;
- Hepatitis A virus is more common in areas that lack adequate sanitation or have poor hygiene practices;
- Food contaminated by a food handler and contaminated before distribution;
- Infective dose 10-100 viral particles;
- Acid tolerant;
Introduction - Prevalence

- EU notification rate declining last 15 yrs:
  - 1997 -> 14/100.000
  - 2010 -> 2,6/100.000

- US notification rate:
  - 1995 -> 12/100.000
  - 2010 -> 1/100.000

- Seasonal pattern – PEAK IN AUTUMN!

- Between 2007 and 2012, EFSA and ECDC reported 14 outbreaks;

- The food vehicles responsible were fish and seafood products, sandwiches, vegetables, juices, semi-dried tomatoes, bakery products and other foods such as minimally processed food products
Introduction – Survival in food and FCM’s

- Heat stable (relatively-when dried can withstand 10 min at 80°C);
- Viable at least 1 month after exposure to 4°C
- Viable 1 year after exposure to < -18°C
- Shellfish exposed to about 70°C for 47 sec to avoid toughening the meat. However, this is not enough heat to destroy HAV; Immersion of shellfish in boiling water for 3 min is recommended for destroying HAV;
- Persist for a month or more dried on paper, cloth, plastic, aluminum, and ceramics due to lack of lipid envelope;
- Drying often increases viral resistance to other stresses such as heat and sanitizers;
Introduction – Detection challenges

- Detection accomplished by:
  - viral propagation in cell culture,
  - radioimmunoassay,
  - immunofluorescence,
  - plaque assay;

- PCR which identifies viral RNA in a sample; This technique may demonstrate that a virus has been present in a sample but the it may no longer be infectious since the protein coat could be denatured.
Aim

- To determine LOD and recovery for HAV in sliced meat product using qPCR
**Materials and Methods - I**

- CRM, 4 log RNA copies/mL;

- Sliced ham samples were gamma irradiated with 25 kGy Cobalt-60. Afterwards, these were contaminated with ten-fold dilution of respective aqueous solution of HAV and let stand until full absorption;

- Virus elution has been performed by PEG/NaCl precipitation whereas extraction of viral RNA was completed using Trizol/Chloroform method;

- Reverse transcription and one-step qPCR (RT-qPCR) were used to detect cDNA of HAV’s RNA;
QPCR reaction was validated to be sensitive and robust out to 35-36 cycles (1 to 10 HAV genome copies), but products detected beyond 36 cycles were poorly reproducible and generally discarded;

Limit of detection (both 50% and 95%) was established in each run as the sensitivity of the QPCR at 35 or 36 cycles backcalculated to the original starting material;
Results - 1
## Results - Ib

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<th>Well Type</th>
<th>Threshold (dR)</th>
<th>Ct (dR)</th>
<th>Avg Ct</th>
<th>ΔCt</th>
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### Standard curve of HAV decimal dilutions

The graph shows a linear relationship between concentration and log Ct. The equation is:

\[ y = -1.589x + 40.464 \]

with an R² of 0.9993. This indicates a strong correlation between concentration and log Ct values.
Results - II

- Recovery: ca. 46% (combined matrix effect, three independent rep. studies, Level 3 inoculum);
- LOD50% = 211 cp/g of ham;
- LOD95% = 554 cp/g of ham;
Conclusion

- The results of this study show that more efforts to improve elution methods are still needed to detect HAVs in meat products containing viruses in low copies.
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THANK YOU FOR YOUR ATTENTION!