

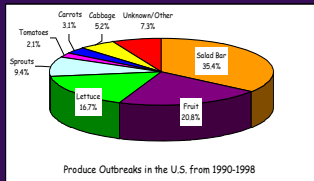
# Presence and Detection of Enteric Viruses in Fresh Produce

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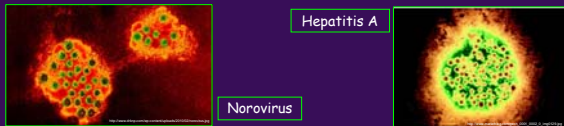


## Introduction

- According to the CDC, more than 50% of foodborne disease outbreaks are caused by Noroviruses (NV), and about 5% are caused by Hepatitis A Virus (HAV)
- Of 2145 outbreaks from 1998-2007, in which Norovirus was confirmed as the etiologic agent, 13.5% were due to fresh produce
- Produce can become contaminated with Enteric viruses via contaminated water sources before harvest, or infected food handlers during preparation and/or packaging
- Enteric viruses are able to survive very well on surfaces and NV and HAV are found to be highly resistant to heat, pressure, temperature and disinfectants (2)
- In a recent market survey of produce items collected from 14 states from March 2008 to February 2009, bacterial and viral indicators such as E.coli, F+ phage and Somatic phage were enumerated
- Types of produce examined included: cilantro, green onion, sprouts, baby spinach, strawberries, carrots, lettuce and other assorted vegetables
- In this study, 208 produce eluates from the previous market survey were examined for NV groups I and II, and HAV



## Norovirus and Hepatitis A



- Human Norovirus strains are classified into three genogroups (I, II, and IV) containing 25 genotypes
- Norovirus Group II (particularly GII.4) is the most common type of Norovirus implicated in foodborne illness
- Two types of hepatitis are enterically transmitted (A and E), HAV has only one serotype which means after infection, immunity is life-long
- NV and HAV are transmitted via fecal-oral routes which include person-to-person contact, and contaminated food or water
- Infective dose: estimated to be 10-100 particles for HAV and NV.
- NV causes acute gastroenteritis: sudden onset of vomiting and diarrhea lasting 2-3 days
- HAV causes fatigue, fever, nausea, diarrhea, weight loss and jaundice

## Methods

### Viral RNA Extraction

- Extracted from PEG precipitates of eluates of 208 produce samples using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA)
- Extract positive controls of NVGI, NVGII, and HAV

### Inhibition Evaluation

- Extracted positive controls were seeded into each produce type to determine the level of inhibition occurring from co-concentrates
- Level of inhibition evaluated with reverse transcriptase quantitative polymerase chain reaction (RT-qPCR)

### RT-qPCR

- Amplify extracted produce samples using NV and HAV specific primers and RT-qPCR protocols to discover whether or not the produce samples carried detectable levels of the viruses

- Evaluate  $C(t)$  graphs to identify samples which are presumptively positive

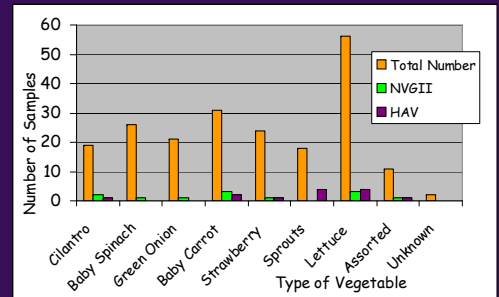
### Confirmation of Positives

- Run presumptive positives on a gel to visualize banding with gel electrophoresis
- Confirm with sequencing

## Results

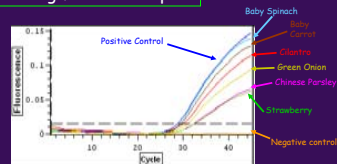
- Twelve of the 208 produce samples appeared presumptively positive for NVGII and 13 appeared presumptively positive for HAV
- Electrophoresis visualization of the presumptive positives showed high levels of inhibition and in some cases multiple banding or streaking

Distribution of Samples and Number Presumptively Positive for HAV and NV GII



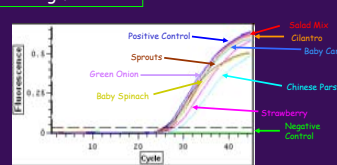
## Results

### Seeding for NV Group II



Vegetable Type	$\Delta C(t)$	% Change
Green Onion	1.4125	62.43%
Chinese Parsley	3.6115	91.82%
Baby Spinach	0.2625	16.64%
Baby Carrot	0.386	23.48%
Strawberry	5.884	98.31%
Cilantro	1.1825	55.94%

### Seeding for HAV



Vegetable Type	$\Delta C(t)$	% Change
Solid Mix	1.2515	58.00%
Green Onion	0.389	23.63%
Chinese Parsley	2.879	86.41%
Baby Spinach	1.134	54.43%
Sprouts	0.0645	4.37%
Baby Carrot	0.771	41.40%
Strawberry	1.834	71.95%
Cilantro	0.9895	49.60%

What is  $\Delta C(t)$ ?

This is the difference between the average cycle threshold ( $C(t)$ ) of the sample (for example Cilantro) and the average  $C(t)$  of the positive control.

## Discussion and Conclusion

- The produce samples appear to be inhibited from possible co-concentrates
- Presumptive positives found in lettuce and in baby carrots are consistent with the known distribution of vehicles in Norovirus outbreaks
- Further confirmation and analysis of inhibition and produce samples is required
- Produce that is minimally processed or consumed fresh can act as a vector of transmission for foodborne viruses
  - Because produce is typically eaten raw, it is an important source for foodborne illness

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Scientific Methods Inc. for providing the PEG precipitates of the 208 produce samples

## References

1. Glass, R.I., Parashar, U.D., Estes, M.K. (2009) Norovirus Gastroenteritis. *The New England Journal of Medicine* 361, 1176-85.
2. Guaranant, E., Bressard, J., Houde, A., Simard, C., Trothier, Y. (2006) Development of an extraction and concentration procedure and comparison of RT-PCR primer systems for the detection of hepatitis A virus and norovirus GII in green onions. *Journal of Virological Methods* 134, 130-135.
3. Seymour, I.J., Appleton, H. (2001). Foodborne Viruses and Fresh Produce. *Journal of Applied Microbiology*, 91, 759-773.