The Role of the Public Health Laboratory in Assessing Norovirus Diversity and Potential Impact of Genetic Variations in Local Outbreaks

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Abstract

Introduction:
A total of 192 suspected norovirus (NoV) specimens linked to 24 reported outbreaks were analyzed during 2006–2011 from long-term care facilities, hospitals, area restaurants and social events.

Methods:
Nucleic acid was extracted from stool samples and tested for NoV RNA using CDC developed real-time RT-PCR assay targeting highly conserved ORF1-ORF2 junction regions of the NoV genome *(Fig 1)*. Subsequent sequence analysis was carried out to determine genetic variations *(Fig. 2)*.

Results:
A total of 87 of 192 (45.3%) specimens were positive for NoV Genogroup II (GII) by real-time PCR assay *(Fig. 3)*. Sequence analysis of C and D regions of capsid gene identified variant GII.4 Minerva as the predominant circulating strain, followed by two minor outbreaks caused by GII.4 New Orleans and GII.6. Genogroup diversity was detected within all identified GII outbreaks (GII.3, GII.6, GII.4 & GII.12) by pairwise multiple sequence alignment using a neighbor joining tree. Data from the CDC CaliciNet confirmed the circulating strains in SE Wisconsin during the time period.

Conclusions:
NoV ability to spread and co-circulate with other variants increases the potential likelihood of genetic recombination resulting in novel viral subtypes. Active epidemiology and laboratory investigation of suspected NoV outbreaks are essential to monitor the genetic variation of the circulating strains, incident cluster analysis, and source tracking during local and regional outbreaks.

Materials and Methods

Figure 1: Norovirus Regions Targeted by Detection and Typing RT-PCR Assays

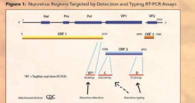
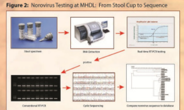


Figure 2: Norovirus Testing at MHDL: From Stool Cup to Sequence



Introduction

- Family *Caliciviridae* – ssRNA, non-enveloped, 26-34 nm viruses
- 4 genera: *Lagovirus, Vesivirus, Sapovirus, Norovirus*
- 5 *genogroups:* GI, GII, GIV (humans), GIII (pigs and cows), & GV (mice)
  - ≥ 25 *genotypes (genetic clusters)*
  - Ongoing emergence (drift) resulting in new *strains* among *genotypes*
  - GII.4 *strains* currently predominate worldwide
- Virus typically circulates during the winter season (September–March)
- Virus infects all age groups: Morbidity/mortality is acute in children under 5, elderly, and immunocompromised people
- According to CDC, approximately 50% of all GI-related outbreaks are caused by Norovirus[1] *(Fig. 4)*.

Figure 3: Specimens Tested for NoV by MHDL 2006–2011

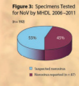
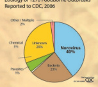


Figure 4: Confirmed and Suspected Etiology of 1270 Foodborne Outbreaks Reported to CDC, 2006



Norovirus Transmission

- Person-to-person, food and/or water, contact with contaminated objects
- Illness typically begins 12–48 hours post infection with symptoms such as acute onset of diarrhea, vomiting, nausea, abdominal cramps, and low-grade fever
- Disease lasts 1–3 days and virus can be shed for 2–5 days after infection

Norovirus Evolution

Norovirus experiences genetic drifts with increasing frequency:

- Evolution is indicative of immune driven selection caused by inadequate population immune response to the virus[2]
- Successful co-circulation of GII.4 with other genotypes indicates that evolutionary pressure does not affect all variants equally[3]

Co-circulating genotypes might be linked to specific transmission/outbreak settings[3]:

- Multi-year study shows that GII.4 genotype highly linked to person-to-person outbreaks in closed-transmission settings such as long-term housing, hospitals, and residential institutions
- Non GII.4 genotype variants were linked to foodborne outbreaks in settings such as social events and restaurants

Study Findings

Norovirus Surveillance in Wisconsin

- No typical seasonality established for NoV, however MHDL shows October to May outbreak incident trend, similar to CDC data[4] *(Fig. 5a and 5b)*.

Figure 5a: Seasonality of Norovirus Detection

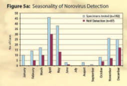
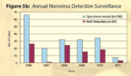
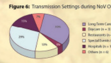


Figure 5b: Annual Norovirus Detection Surveillance



- Transmission settings show that outbreaks occurred more often in clinical or person/patient care settings (51%).
- Special events and restaurants provide a significant number of specimens, however those were sporadic and self-limiting outbreaks *(Fig. 6)*.

Figure 6: Transmission Settings during NoV Outbreaks



- Evolution of NoV genogroup diversity during 2006 – 2011
- Predominant outbreak genogroup was GII.4, with the emergence of Minerva and New Orleans variants worldwide

Figure 7: NoV Surveillance MHDL 2006 – 2011

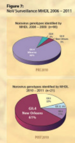


Figure 8: Wisconsin NoV Surveillance WSLH

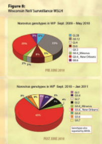


Figure 9:
Norovirus Diversity Reported by WSLH 2009 – 2011

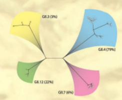
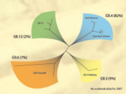


Figure 11:
Norovirus Diversity Reported by MHDL 2006 – 2011



- Norovirus diversity and genetic variations in local gastrointestinal outbreaks

Figure 10: 2009 – 2011 NoV GII.4 Genotype Diversity Reported by WSLH

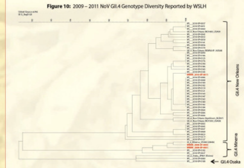
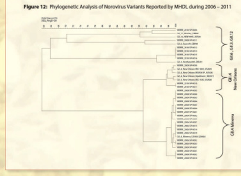


Figure 12: Phylogenetic Analysis of Norovirus Variants Reported by MHDL during 2006 – 2011



Norovirus diversity in Wisconsin (2006- 2011)

Phylogenetic analysis indicates NoV GII.4 is the predominant genotype circulating in Wisconsin during 2006–2011

- GII.4 Minerva 2006–2009 (MHDL retrospective study)
- GII.4 New Orleans 2009–2011 (MHDL & WSLH cumulative reports)
- Additional GI and non-GII.4 genotypes co-circulated and caused ~20% of all outbreaks reported during that time period

MHDL and WSLH have reported significant similarities in NoV genotype outbreaks during 2009- 2011

- NoV diversity reported by WSLH during 2009–2011 (CaliciNet data[6]):
  - Majority of outbreaks reported by WSLH were caused by GII.4, while other non GII.4 successfully co-circulated during the same period *(Fig. 9)*
- GII.4 New Orleans is the most widespread outbreak genotype:
  - Phylogenetic diversity is observed within the GII.4 New Orleans group
  - Mutations indicate not all outbreaks were caused by the same virus
  - Rich diversity in co-circulating genotype increases likelihood of recombination and evolution of new genotypes *(Fig. 10)*
- NoV diversity reported by MHDL during 2006– 2011 *(Fig. 11)*
- GII.4 Minerva is the predominant outbreak genotype:
  - Approx. 80% of outbreaks were related to GII.4 genotype
  - MDHL identified 7 distinct GII.4 Minerva variants during 2006–2009
  - MDHL identified 2 GII.4 New Orleans, 1GII.3, 1GII.6, and 1GII.12 variants in 2010 *(Fig. 12)*

Conclusions

1. Emergence of two predominant outbreak GII.4 genotypes, 7 distinct GII.4 Minerva variants during 2006–2009, and 2 GII.4 New Orleans variants during 2009–2011 in Wisconsin
2. Successful co-circulation of strains indicates evolutionary pressures might not affect all genotypes equally and evolution might be linked to outbreak and/or transmission settings
3. Person-to-person transmission and clinical settings more commonly linked (51%) and persistent, whereas variants from foodborne outbreaks events contributed significantly to NoV diversity, but were sporadic and self-limiting
4. Rich diversity in co-circulating strain within a genotype increases likelihood of recombination, co-infection, and potential evolution of new genotypes
5. Rapid spread of new GII.4 genotypes likely to be linked to insufficient herd immunity response to new viruses and an increase in susceptibility to infection[1]

Critical PH Lab- Epidemiology Study Areas:

1. Monitoring suspected NoV outbreaks and herd immunity is crucial to control of virus spread and community protection
2. Studies linking transmission modes and outbreak settings can help identify potential sources
3. Molecular surveillance of NoV outbreaks increases the likelihood of detecting phylogenetic relationships amongst emerging genotypes
4. Timely recognition of new genetic variants allow for more adequate patient treatment and ensures prompt public health response during local outbreaks

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- CaliciNet team: Leslie Barclay, Nicole Gregoricus, Jan Vinje

References

1. Patel, *et al.* 2009. J. Clin Virol, 44:1-8
2. Brown, *et al.* 2008. The Evolution of Norovirus, The Gastric "Flu" PLOS, 5-2: 187-189
3. Kroneman, *et al.* 2008. J Public Health, 30 (1): 82-90
4. MMWR. 2011: 60(RR03);1-15
5. WSLH reports: http://www.slh.wisc.edu/outreach-data/past-event-detail.php?id=200
6. CaliciNet: http://www.cdc.gov/foodsafety/activities.html

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