

Mapping Viral Gastroenteritis Outbreaks: Terra Incognita

Xiaoli Lilly Pang, Ph.D.
Bonita E Lee, MD MSc (Epi)

Provincial Public Health Laboratory
University of Alberta
Edmonton, Alberta, Canada

PROVLAB

Outline

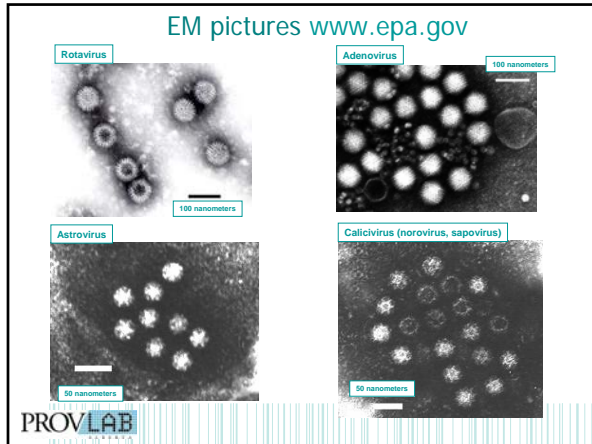
- ♦ Enteric viruses – clinical features and enhanced detection using real-time nucleic acid amplification tests
- ♦ Epidemiology of viral gastroenteritis outbreaks in Alberta – the known and uncharted territories
- ♦ Characterization of norovirus strains – missing link to outbreaks burden?
- ♦ What is in the horizon?

PROVLAB

Background

- ♦ Viral gastroenteritis is a very common illness and causes significant morbidity and mortality and remains as one of the top 10 cause of death in low-income countries
- ♦ The viruses commonly associated with gastroenteritis are known as “enteric viruses”, including:
 - **RNA virus:** norovirus, rotavirus, astrovirus and sapovirus
 - **DNA virus:** adenovirus 40 and 41

PROVLAB



Real time RT-PCR for enteric viruses

- A real time RT-PCR assay was developed and implemented for Norovirus testing in GIO in 2004 (Pang et al. 2005 JCV)
- A multiple-target real time PCR panel (mtrt-PCR) was developed and validated for all five enteric viruses

TagMan probes

ABI PRISM 7500 Sequence Detection System

Amplification curve

PROVLAB

Enteric virus & GIO

- Norovirus is the most common cause of non-bacterial gastroenteritis outbreaks (GIO) with worldwide epidemics reported since 1995

	No. of GIO	Noro	Rota	Sapo	Enteric Adeno	Astro	
USA	226 in 2000 - 2004	81%		2%			Blanton LH JID 2006
Netherlands	941 in 1994 - 2005	78%	4.9%		1.0%	0.5%	Siebenga JJ Emerg Infect Dis 2007
Norway	204 in 2000-2005	91%					Kirsti V JCM 2006

- The role of other enteric viruses in GIO are not as well known and there were only occasional reports on their incidence

PROVLAB

FORT LAUDERDALE, FL - More than 700 passengers and crew were stricken with Norovirus during the 16-day trans-Atlantic voyage. POSTED: 10:33 am EST November 19, 2006



Outbreak Investigations in Alberta

- ◆ Provincial Public Health Laboratory in Alberta works in partnership with Public Health Investigators to investigate all types of outbreaks
- ◆ Every outbreak is assigned an EI# and the microbiologist/virologist acts as the laboratory point person and the external investigator can use the EI# to track laboratory results of the submitted samples



Provincial Laboratory for Public Health Intranet - Portal Home - Microsoft Internet Explorer

Public Health & EAHS microbiology

Outbreak ID	Reported	Date	Facility	Point Person	Senior Analyst	Microbiologist
012005-01	01	13-Jan-05	...	Kennedy, Dr. Robert	Norman-like Agent	...
012005-02	02	14-Jan-05	...	Tymall, Dr. Greg	Norman-like Agent	...
012005-03	03	14-Jan-05	...	Louis, Dr. Marie	Norman-like Agent	...
012005-04	04	13-Jan-05	...	Tymall, Dr. Greg	Influenza A Virus	...
012005-05	05	13-Jan-05	...	Faloutsos, Dr. Kevin	Influenza A Virus	...
012005-06	06	13-Jan-05	...	Faloutsos, Dr. Kevin	Norman-like Agent	...
012005-07	07	13-Jan-05	...	Faloutsos, Dr. Kevin	Rotavirus	...
012005-08	08	13-Jan-05	...	Faloutsos, Dr. Kevin	Norman-like Agent	...
012005-09	09	13-Jan-05	...	Tymall, Dr. Greg	Norman-like Agent	...
012005-10	10	13-Jan-05	...	Louis, Dr. Marie	Influenza like agent	...
012005-11	11	13-Jan-05	...	Tymall, Dr. Greg	Norman-like Agent	...
012005-12	12	13-Jan-05	...	Louis, Dr. Marie	Unknown	...
012005-13	13	13-Jan-05	...	Tymall, Dr. Greg	Norman-like Agent	...
012005-14	14	13-Jan-05	...	Faloutsos, Dr. Kevin	Norman-like Agent	...

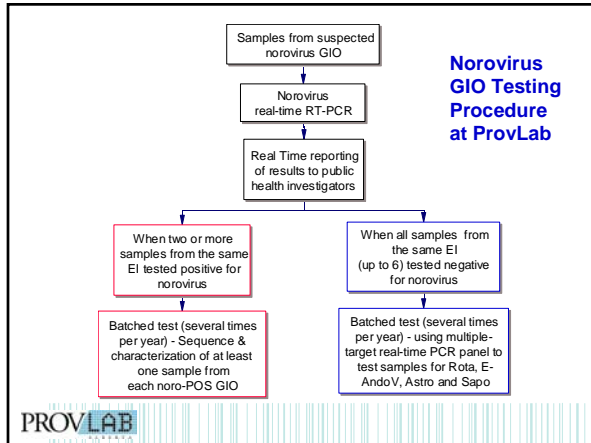
Records 1 to 13 of 148

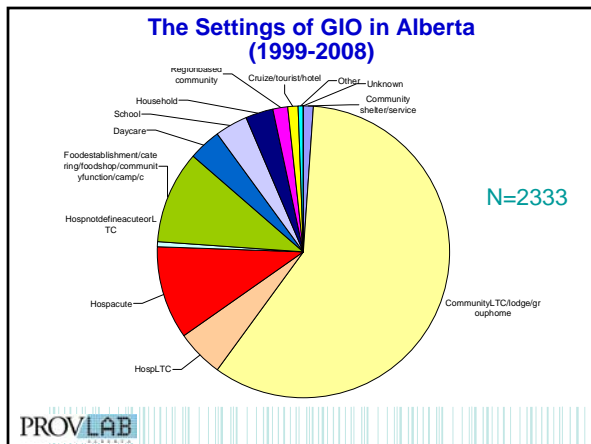


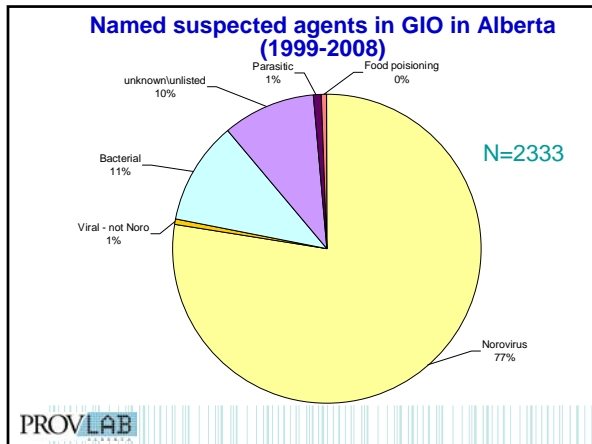
Gastroenteritis Outbreaks in Alberta

- ◆ Since 1999, conventional RT-PCR was used to identify norovirus. In March 2004, a highly sensitive and specific real-time RT-PCR assay that can differentiate norovirus genogroup I and genogroup II was implemented (Pang et al. 2005 JCV)
- ◆ Samples from suspected norovirus outbreaks can be directed for:
 - Routine GIO protocol with set-up of enteric bacterial cultures pending norovirus testing result
 - OR
 - Norovirus detection only

PROVLAB



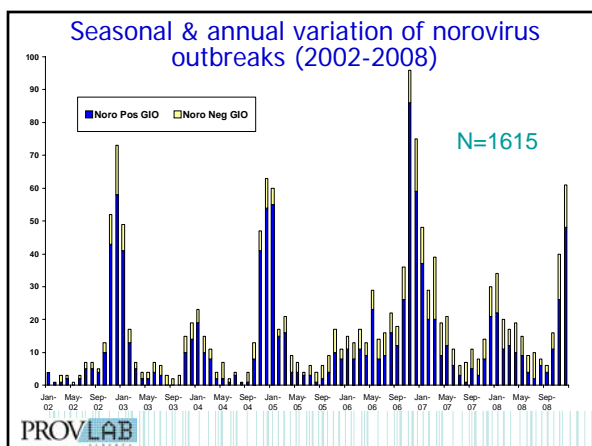


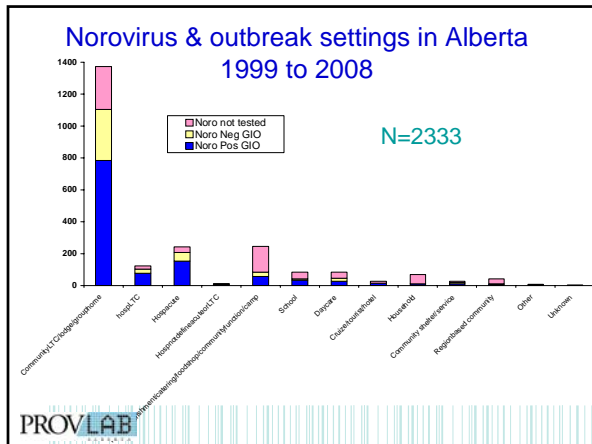


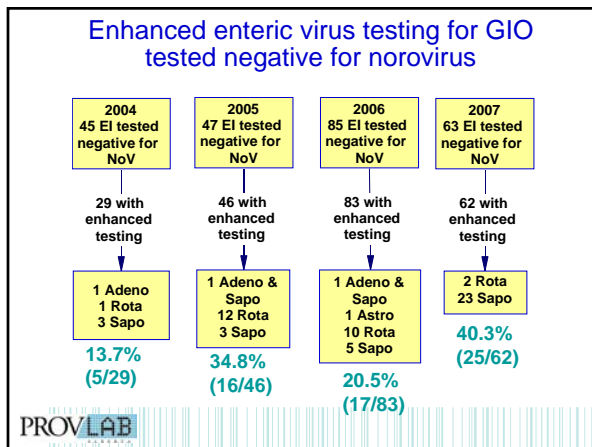
Norovirus Outbreaks in Alberta

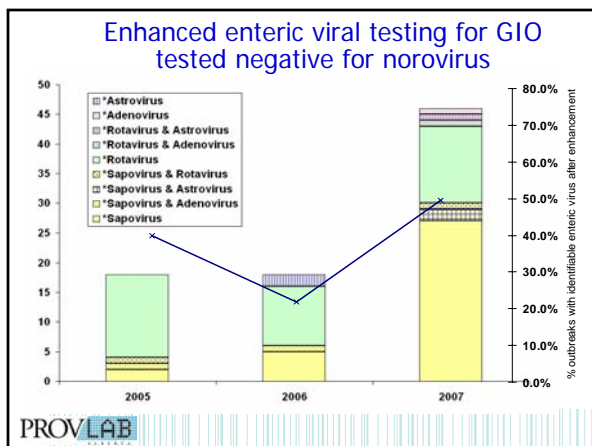
- ♦ From 1999 to 2008, samples from 1,650 (71%) GIO were submitted for norovirus testing, and one or more samples from 1,171 GIO tested positive for norovirus (71%)
- ♦ The highest incidence of NGO occurred in the winter with a pattern of high epidemic activity every two years.

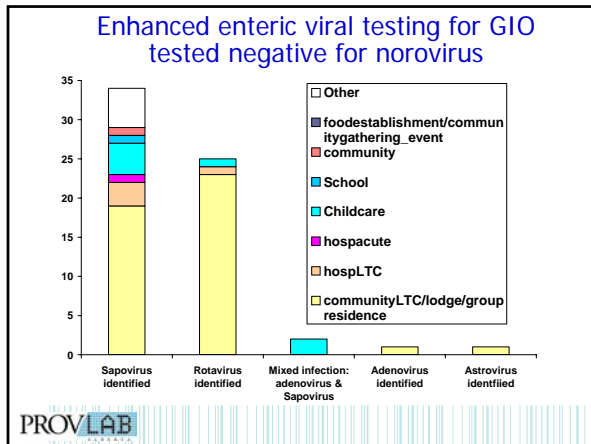
PROVLAB











Summary

- ◆ Public health investigators are very good in identifying which GI outbreaks are caused by viruses
- ◆ Norovirus is the most common cause of GIO and up to 71% outbreaks were caused by norovirus in the last 9 years in Alberta
- ◆ With enhanced testing for enteric viruses other than norovirus in noro-negative GIO, etiological agents were identified in 29% of GIO with enhancement
- ◆ Both rotavirus and sapovirus play an important role in GIO in Alberta

PROVLAB

Genetic Analysis of Norovirus Strains from Outbreaks in Alberta

What is the mechanism behind the seasonal and annual variation of norovirus outbreak?

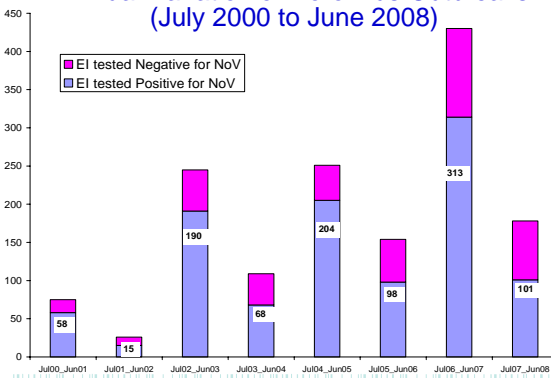
PROVLAB

Background and objective

- Recent studies reported that genetic variants of norovirus capsid GII.4 have rapidly evolved to novel epidemic strains causing up to 80% of all norovirus GIO in US and Europe, but there is no molecular epidemiology data available to describe the trend in Alberta.
- This study is to identify the genetic drift of GII.4 and the new variants associated with epidemic norovirus outbreaks in the last 8 years in Alberta.

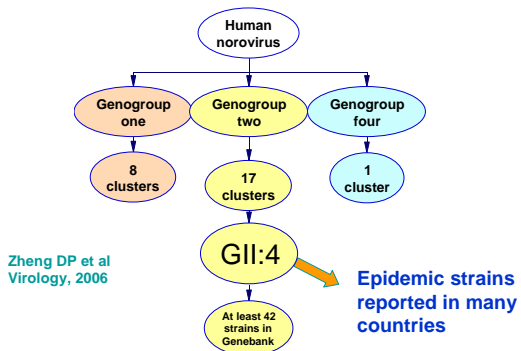
PROVLAB

Annual Variation of Norovirus Outbreaks (July 2000 to June 2008)



PROVLAB

Norovirus classification



Zheng DP et al
Virology, 2006

PROVLAB

GII.4 Variants

PROVLAB

Methods

- ♦ At least one positive specimen from each confirmed norovirus outbreak was sequenced.
- ♦ The targeted regions for sequencing are: region B (polymerase gene), region E and region D (capsid gene).
- ♦ The sequence data were compared with reference sequences of the genotypes GII.1 to 16 in GenBank using the multiple alignments (BioNumerics software v5.1).
- ♦ Archived sequences of GII.4 were aligned with the reference strains of GII.4 variants 1996, 2002, 2004, 2006a, 2006b and 2008.

PROVLAB

Reference GII.1 to GII.16 strains used in this study for phylogenetic analysis of norovirus from GIO in Alberta

*Zhang et al. Virology, 2006

PROVLAB

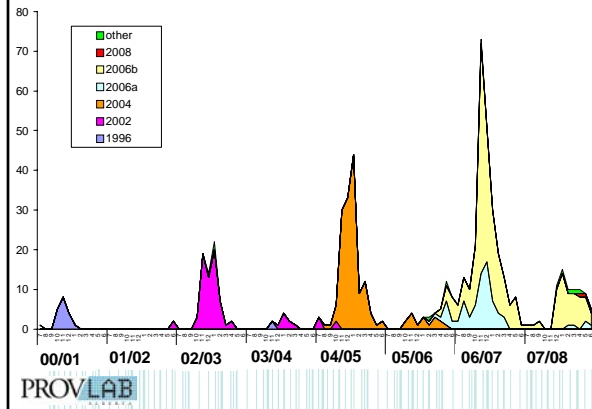
*GII genotypes	Name of strain	Accession number in GeneBank
GII.1	Hawaii/71/US	U07611
GII.2	Ina/02/JP	AB195225
GII.2	Snowmount/76/US	U75682
GII.3	Oberhausen455/01/DE	AF539440
GII.3	Toronto24/91/CA	U02030
GII.4	Lordsdale/93/K	X86557
GII.4	Bristol virus/94/UK	X76716
GII.5	Hillingdon/94/UK	AJ277607
GII.5	Hokkaido133/03/J	AB212306
GII.6	Seacroft/90/UK	AJ277620
GII.7	Leeds/90/UK	AJ277608
GII.8	Amsterdam/98/NL	AF195848
GII.9	Virginia207/97/US	AY038599
GII.10	Erfurt-546/00/DE	AF427118
GII.11	Sw918/97/JP	AB074893
GII.12	Wortley/90/UK	AJ277618
GII.13	Fayetteville/98/US	AY113106
GII.14	M7/99/US	AY130761
GII.15	J23/99/US	AY130762

Results of Sequence Analysis in Norovirus Outbreaks in Alberta

	Jul00 to Jun01	Jul01 to Jun02	Jul02 to Jun03	Jul03 to Jun04	Jul04 to Jun05	Jul05 to Jun06	Jul06 to Jun07	Jul07 to Jun08
NoV EI	58	15	190	68	204	98	313	101
sequence (%)	36.2	20.0	42.1.0	44.1	77.5	58.2	87.9	95.1
Genogp 1	0	0	2	16	0	1	14	2
Genogp 2	21	3	78	14	158	56	261	94
GII:4	18	2	68	10	146	41	251	75
% of GII	86	67	87	71	92	73	96	80

PROVLAB

Norovirus GII:4 Variants in GI Outbreaks



PROVLAB

Conclusions

- ♦ GII.4 was the predominant genotype found in norovirus GIO from 2000 to 2008 with a shift in capsid sequence over time.
- ♦ New GII.4 variants were associated with epidemic norovirus outbreaks.
- ♦ Sequence data provides valuable information for identifying new emerging variants of norovirus and is imperative for the prediction of norovirus GIO burden

PROVLAB

Future Horizon

- ♦ Study the evolution and dynamic changes of norovirus GII.4 variants and outbreak burden
- ♦ Determine the relationship between host susceptibility and immunity with the antigenic predominance of circulating norovirus in GIO
- ♦ Investigate herd immunity and the evolution of GII.4 strains to better predict future epidemics

PROVLAB

Acknowledgement

- ♦ Collaborative partners participating in the outbreak investigation
- ♦ Dr. Jutta Preiksaitis
- ♦ All the staff in Provlab in Edmonton and Calgary
- ♦ Sallene Wong & Calgary Molecular Diagnostic Laboratory
- ♦ Staff in Research Laboratory in Edmonton, Provlab
- ♦ AHW enhancement initiatives

PROVLAB
