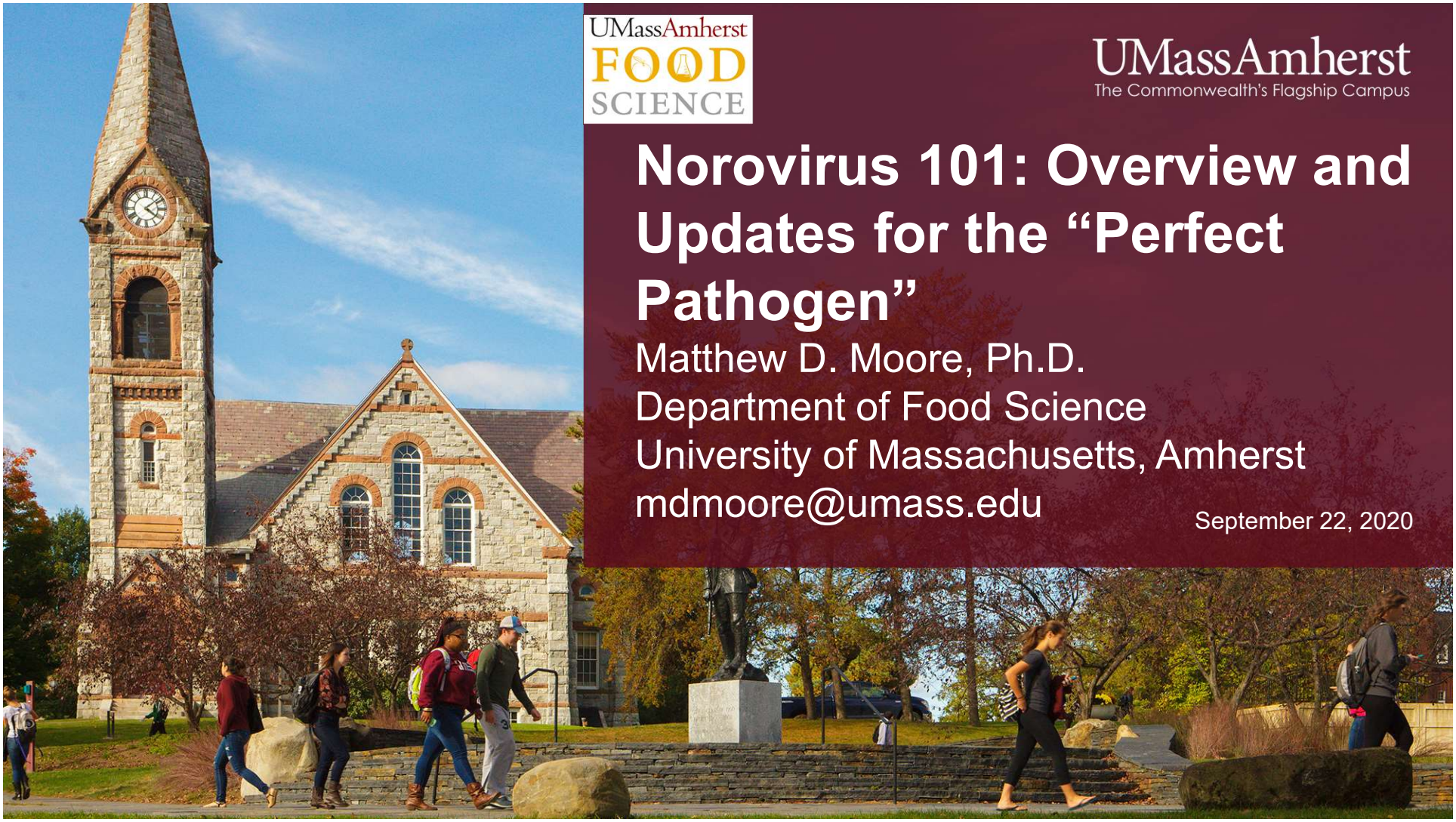

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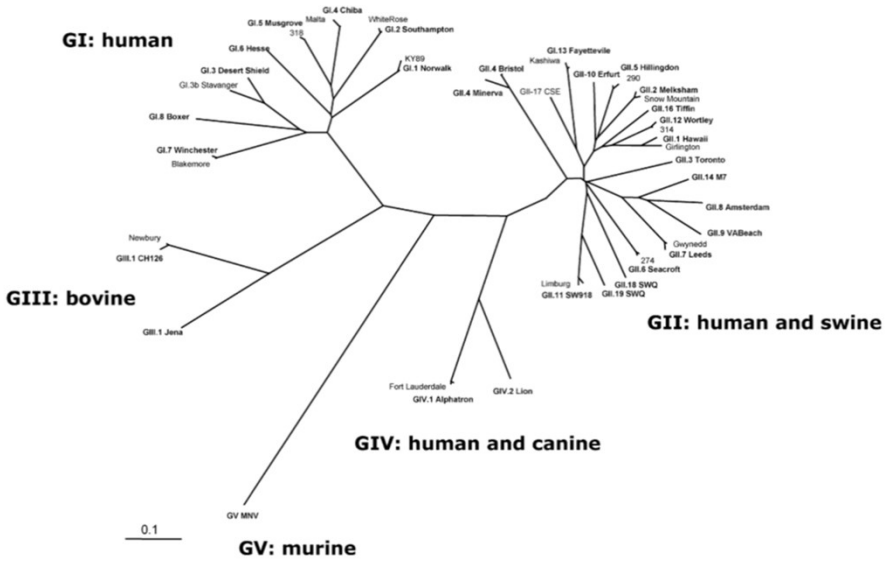
Norovirus 101: Overview and Updates for the "Perfect Pathogen"

Matthew D. Moore, Ph.D.
Department of Food Science
University of Massachusetts, Amherst
mdmoore@umass.edu

September 22, 2020

Human Norovirus

- Not traditionally (prior to 2014) cultivable *in vitro*
- Low infectious dose
- Survives on surfaces
- Disinfection difficult
- Asymptomatic shedding and carriers
- Diversity and rapid evolution
- No long-lasting immunity



(Vinje 2014; Bull et al. 2010; Patel 2009)

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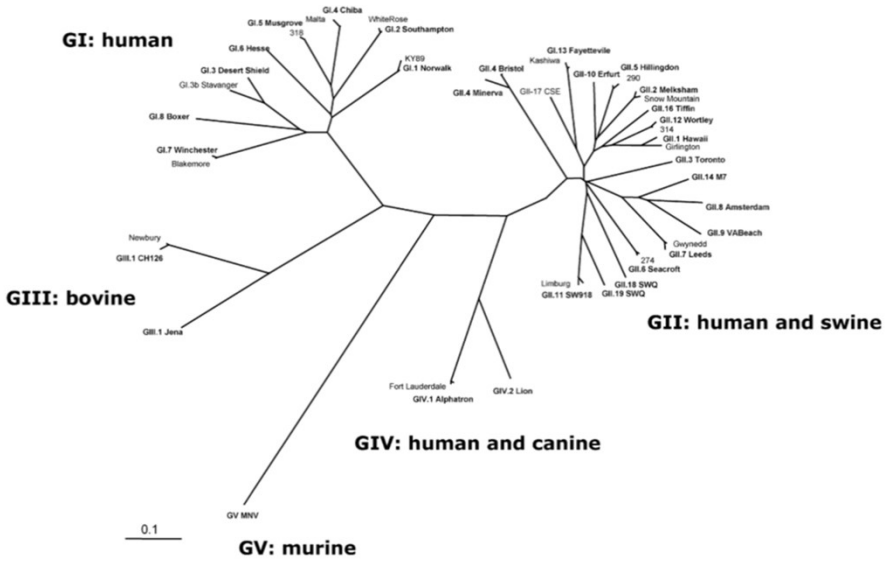

(Vinje 2014; Bull et al. 2010; Patel 2009)

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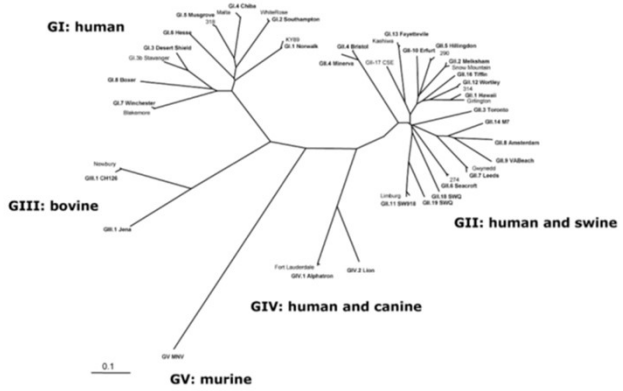
- **Diversity and rapid evolution**
- No long-lasting immunity

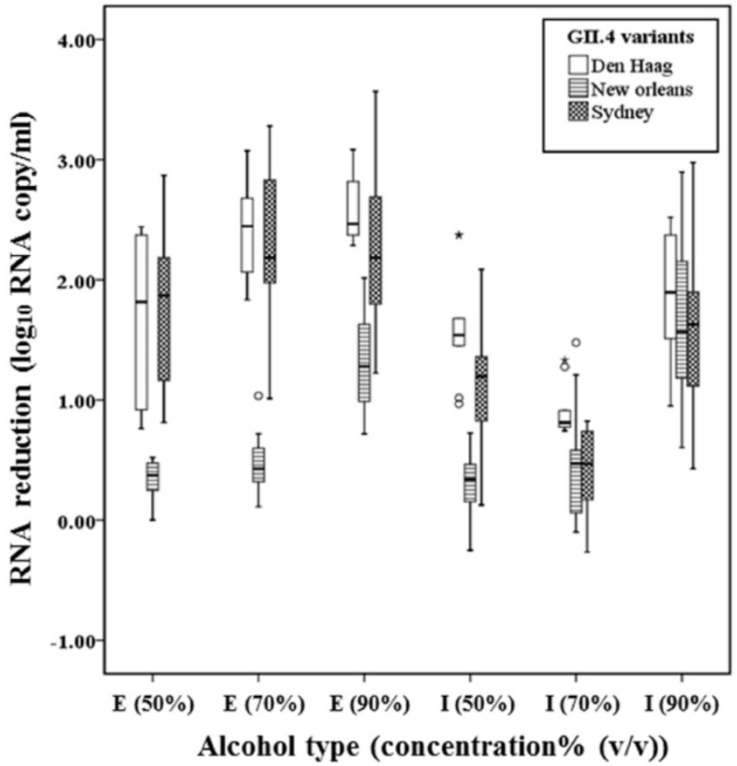
Note: Genogroup (II) → Genotype (4) → Strain (Sydney): GII.4 Sydney



(Vinje 2014; Bull et al. 2010; Patel 2009)

Diversity of Susceptibility

- Differences with GII.4 strains for inactivation



(Park et al. 2016)

Challenges

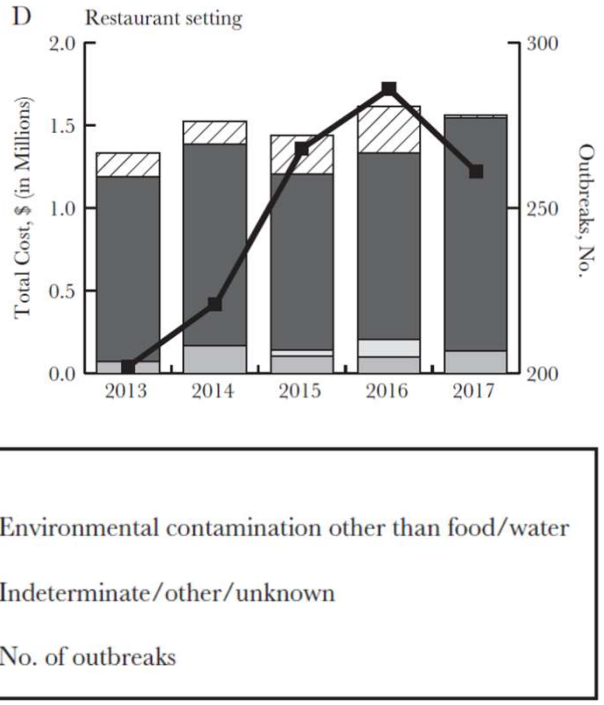
- Resistance to inactivation
  - At currently allowed levels, many agents not effective enough
  - Even heat: 72°C did not remove signal until >10 min

Melting Temperatures Using Aptamer M6-2	
Strain	Melting Temperature (°C)
SYV	73.10 ± 0.43
SMV	75.03 ± 0.80
HOV	68.88 ± 1.10

Melting Temperatures Using HBGA	
Strain	Melting Temperature (°C)
SYV	71.71 ± 0.49
SMV	N/A
HOV	67.69 ± 0.30

The Race for a Different Vaccine

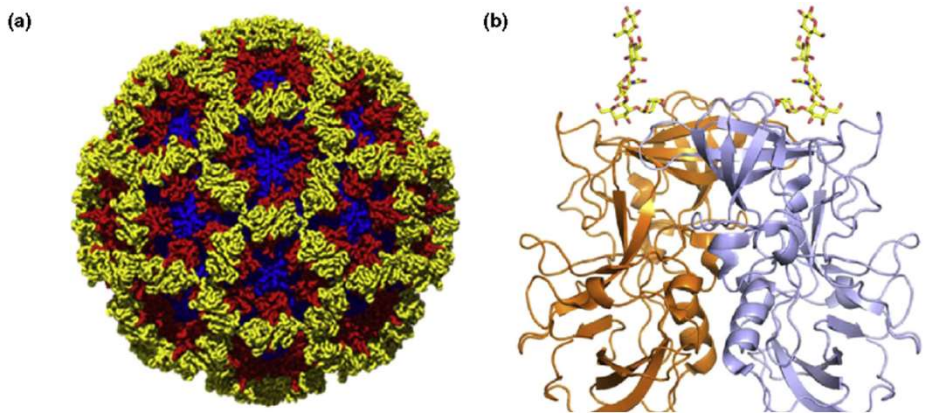
- Alleviate a major burden
  - 900 lives
  - 109,000 hospitalizations
  - 465,000 emergency department visits
  - 2.3 million urgent care visits
  - $430-740 million in healthcare costs alone
  - $10.6 billion overall annually



(Burke et al. 2020; Bartsch et al. 2020)

The Race for a Different Vaccine

- Numerous challenges:
  - Antigenic and biological diversity
  - General lack of immune cross-protection with other genotypes
  - Can be re-infected with same genotype after a few years
  - Immune correlate of protection still a little unclear



(Esposito et al. 2020; Prasad et al. 2016)

The Race for a Different Vaccine

- Virus-like particle (VLP) based vaccines
  - Viral capsid without nucleic acid inside
  - GI.1, GII.3, GII.4 genotypes
  - Some combined with other virus targets
  - Preclinical through Phase II
  - A number of Phase II trials with positive preliminary results
- P particle vaccines
  - The “Protruding” domain of the major capsid protein
    - Outermost portion
    - Involved in receptor binding and major antigenic target
    - Mostly preclinical

(Esposito et al. 2020)

The Race for a Different Vaccine

- Recombinant adenovirus vaccine
  - Uses adenovirus to deliver norovirus major capsid protein
  - Orally administered
  - GI.1 and GII.4
  - Currently in Phase II
  - Positive results for safety as well as robust immune response in animal model

(Esposito et al. 2020)

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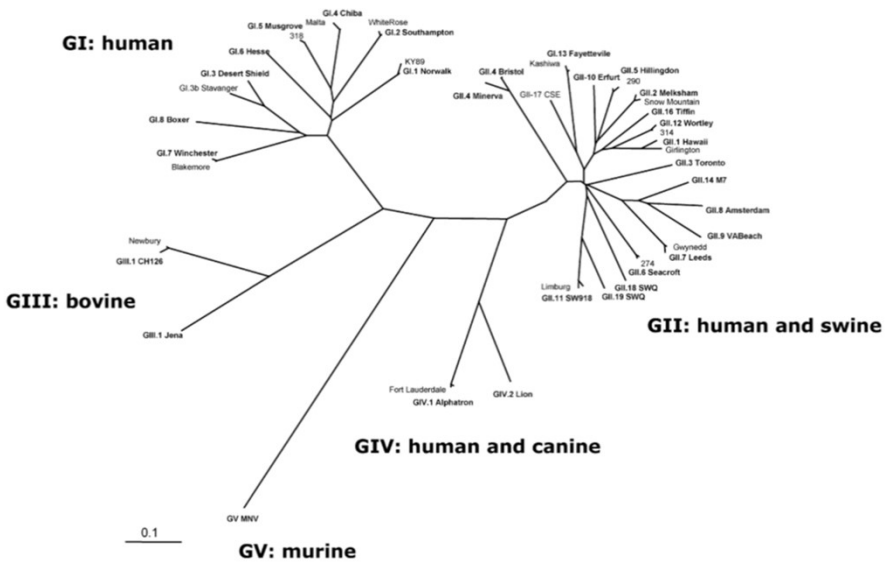


(Vinje 2014; Bull et al. 2010; Patel 2009)

THE Challenge

- *In vitro* cultivation
  - Over 40 years
  - Mid-life crisis: 3D cell culture model

THE Challenge

- *In vitro* cultivation
  - Over 40 years
  - Mid-life crisis: 3D cell culture model

In Vitro Cell Culture Infectivity Assay for Human Noroviruses

Timothy M. Straub,* Kerstin Höner zu Bentrup,† Patricia Orosz-Coghlan,‡ Alice Dohnalkova,*
Brooke K. Mayer,*[1] Rachel A. Bartholomew,* Catherine O.Valdez,* Cynthia J. Bruckner-Lea,*
Charles P. Gerba,‡ Morteza Abbaszadegan,§ and Cheryl A. Nickerson†[1]

(Straub et al. 2007)

THE Challenge

- *In vitro* cultivation
  - Over 40 years
  - Mid-life crisis: 3D cell culture model

OPEN ACCESS Freely available online

PLOS ONE

Challenges of Culturing Human Norovirus in Three-Dimensional Organoid Intestinal Cell Culture Models

Efstathia Papafragkou[1,2], Joanne Hewitt[3], Geun Woo Park[1], Gail Greening[3], Jan Vinjé[1]*

1 Division of Viral Diseases, Center for Disease Control and Prevention, Atlanta, Georgia United States of America, **2** Center for Food Safety and Applied Nutrition, Division of Molecular Biology, Food and Drug Administration, Laurel, Maryland, United States of America, **3** Institute of Environmental Science and Research Ltd, Kenepuru Science Centre, Porirua, New Zealand

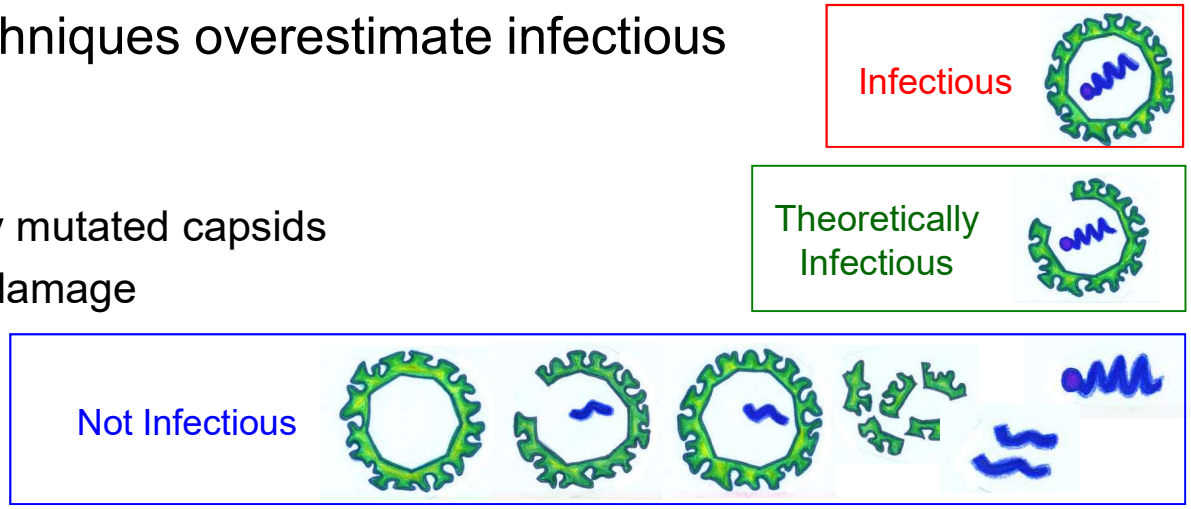
(Papafragkou et al. 2013)

THE Challenge

- *In vitro* cultivation
  - Over 40 years
  - Mid-life crisis: 3D cell culture model
  - Complicates study
    - **Infectivity dilemma**

Infectivity Dilemma

- Lack of widely available/ideal *in vitro* cultivation
  - Cannot follow/examine behavior of infectious particles
  - Implications for inactivation methods, detection, and fundamental study of solution of viral particles
- Genome amplification techniques overestimate infectious particles
  - Free RNA
  - RNA from damaged/fatally mutated capsids
  - RNA with fatal mutations/damage



(Images courtesy Rebecca Goulter)

Cultivable Surrogates

- Utilize related cultivable surrogate viruses that are genetically and/or structurally related/similar
- Feline calicivirus, Tulane virus, murine norovirus (MNV), bacteriophage MS2
- However, differences in susceptibility
  - Possibility they are weaker than human norovirus
- Murine norovirus widely used for pathogenesis
  - *In vivo* and *in vitro*
- Differences in biological presentation

(Richards 2012; Wobus 2018; Karst & Tibbetts 2016)

Murine Norovirus Receptor

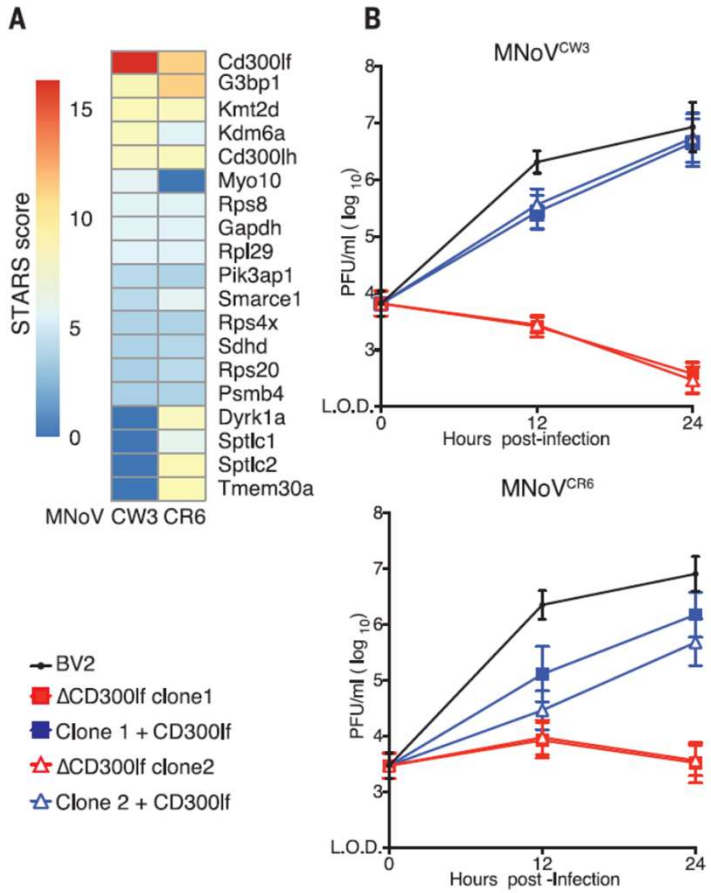
VIROLOGY

Discovery of a proteinaceous cellular receptor for a norovirus

Robert C. Orchard,[1*] Craig B. Wilen,[1*] John G. Doench,[2] Megan T. Baldridge,[1] Broc T. McCune,[1] Ying-Chiang J. Lee,[1] Sanghyun Lee,[1] Shondra M. Pruett-Miller,[3] Christopher A. Nelson,[1] Daved H. Fremont,[1] Herbert W. Virgin[1†]

Murine Norovirus Receptor

- Identify proteinaceous receptors, CD300lf and CD300ld, necessary for cell permissiveness to MNV infection



(Orchard et al. 2016)

Murine Norovirus Receptor

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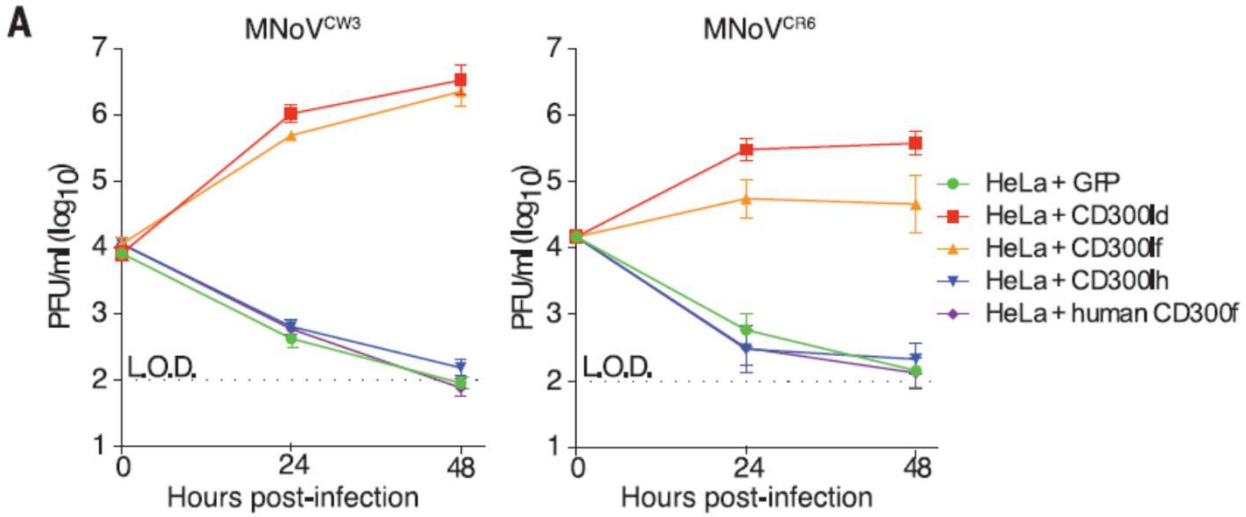


(Orchard et al. 2016)

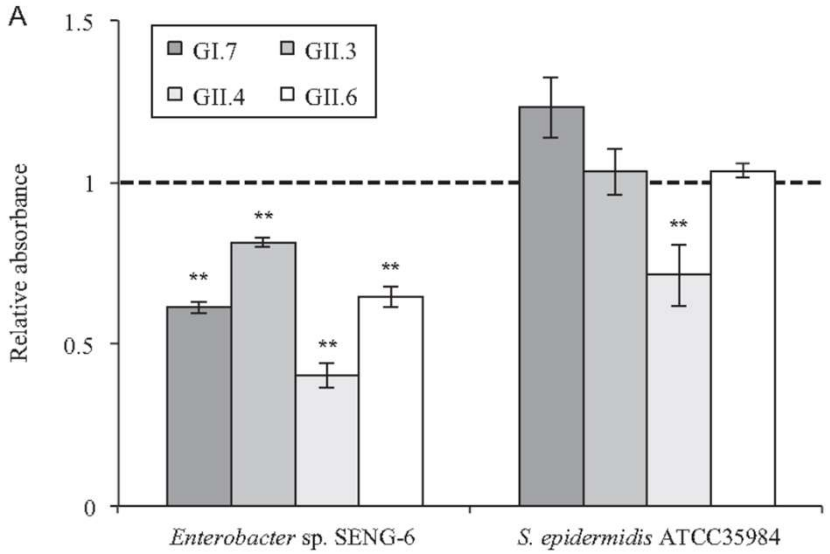
Murine Norovirus Receptor

- Identify functional proteinaceous receptors, CD300lf and CD300ld, necessary for cell permissiveness to MNV infection
- Makes human cells permissive, not vice versa
- Evidence for a small (<5kDa) nonproteinaceous co-factor to facilitate binding
- Not necessarily comparable to human norovirus

(Orchard et al. 2016)

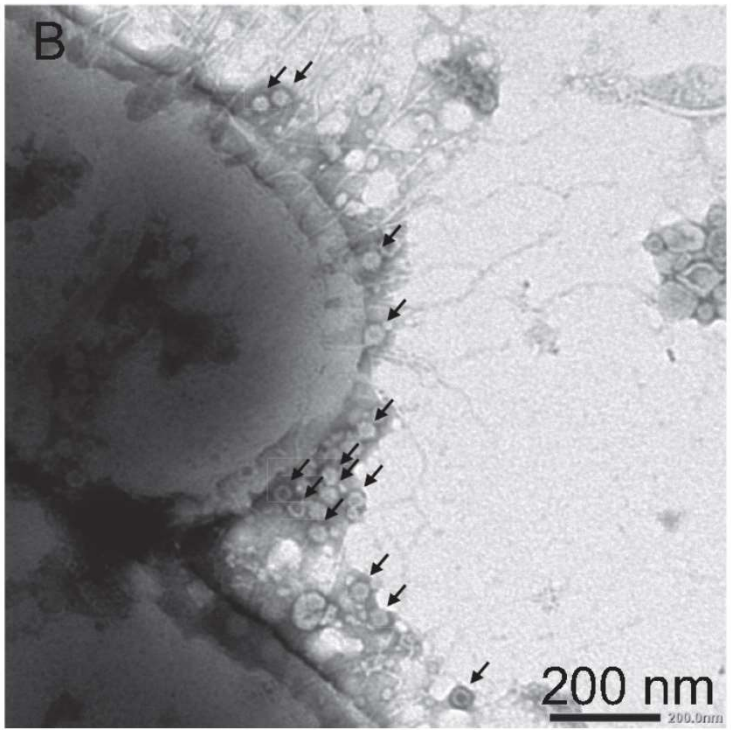
Group Discount?

- *In vitro* cultivation
- Enteric bacteria –Potentially a co-factor?
  - HuNoV found to bind *Enterobacter cloacae*



(Miura et al. 2013)

Group Discount?



(Miura et al. 2013)

Group Discount?

- Enteric bacteria as potential co-factor for infection in human B cells

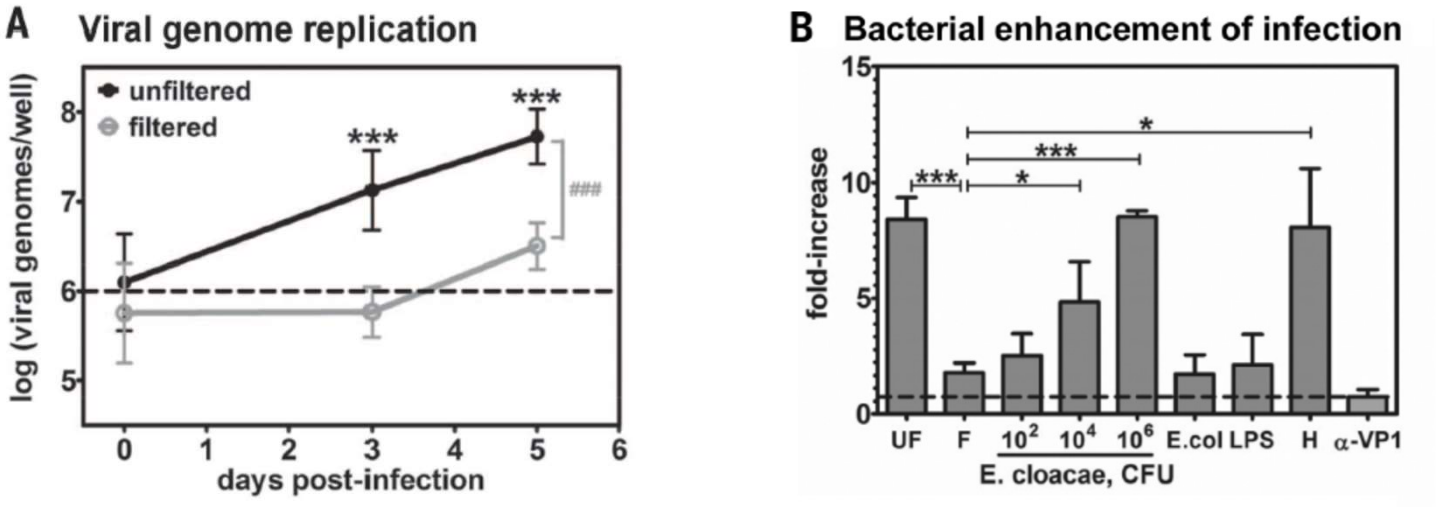
Enteric bacteria promote human and mouse norovirus infection of B cells

Melissa K. Jones,[1*] Makiko Watanabe,[1*] Shu Zhu,[1] Christina L. Graves,[2,3] Lisa R. Keyes,[1] Katrina R. Grau,[1] Mariam B. Gonzalez-Hernandez,[4] Nicole M. Iovine,[5] Christiane E. Wobus,[4] Jan Vinjé,[6] Scott A. Tibbetts,[1] Shannon M. Wallet,[2,3] Stephanie M. Karst[1†]

(Jones et al. 2014)

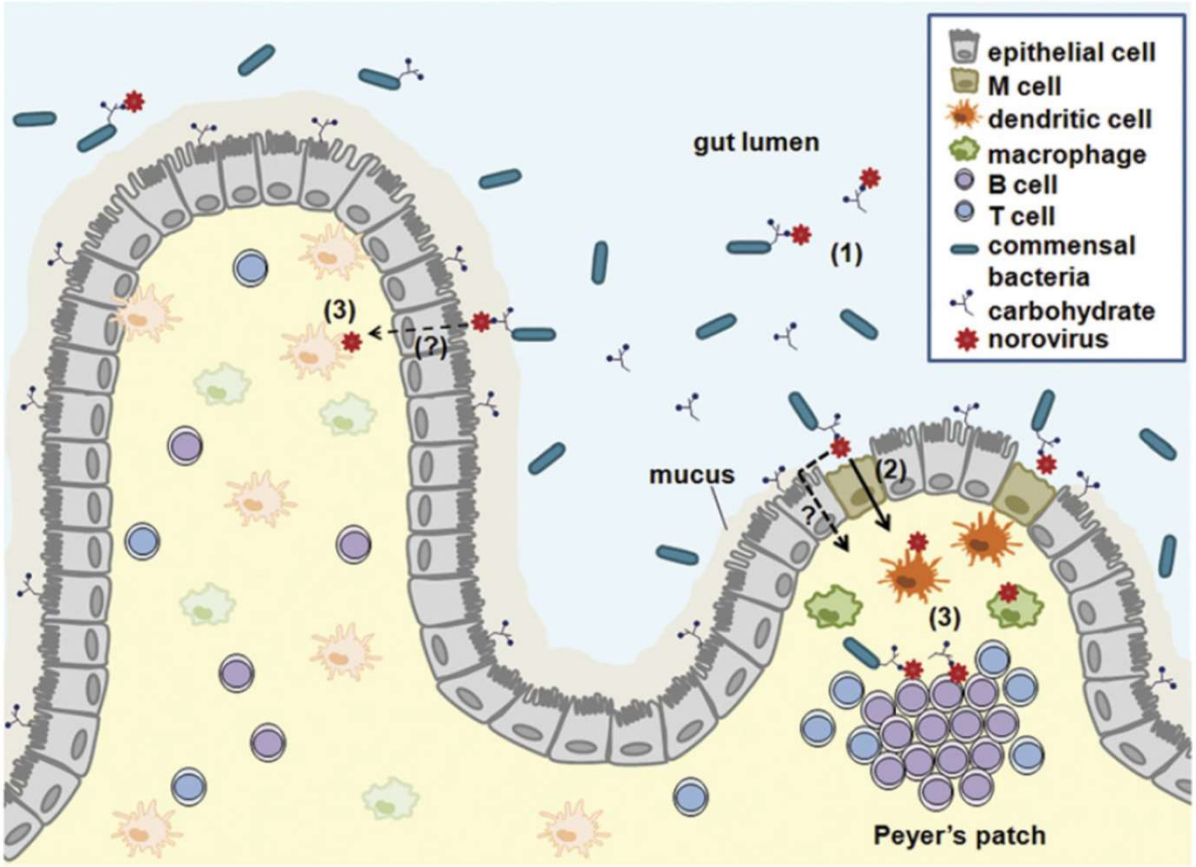
Group Discount?

- Enteric bacteria as potential co-factor for infection in human B cells



(Jones et al. 2014)

Group Discount?



(Karst & Wobus 2015)

Group Discount?

- Enteric bacteria as potential co-factor for infection in human B cells
  - However, can have issues in replicating
  - < 3 $\log_{10}$ production → sensitivity a challenge
  - Requirement of additional co-factors could confound inactivation study
- Antibiotic treatment reduces viral titer in mouse model

(Jones et al. 2015; Almand et al. 2017)

Enteric Bacteria and Norovirus

- Some evidence binding increases stability of virus
  - Though some conflicting reports
- Some bacteria down-regulate by enhancing virus-specific antibody titer
- Others promote viral adherence and binding
- Bacterially modified bile acids (secondary bile acids) may enhance viral infection
  - Can enhance binding to receptor

(Moore & Jaykus 2018; Almand et al. 2017; Neu & Mainou 2020; Jones et a. 2016; Sullender & Baldridge 2018)

Enteric Bacteria and Norovirus

- Commensal bacteria can enhance production of secretory immunoglobulins
  - Actually promotes MNV infection
- Potential bacterial effect on tropism of virus
- Can alter gut microbiota, promote higher *Firmicutes* to *Bacteroidetes* ratio (dysbiosis)
  - How long that lasts unclear
- Native microbiota on produce may aid attachment

(Moore & Jaykus 2018; Almand et al. 2017; Neu & Mainou 2020; Jones et a. 2016; Sullender & Baldridge 2018)

Enter the Enteroids

- Another cell culture model released from group at Baylor
- Involves creation of human intestinal enteroids (HIEs)

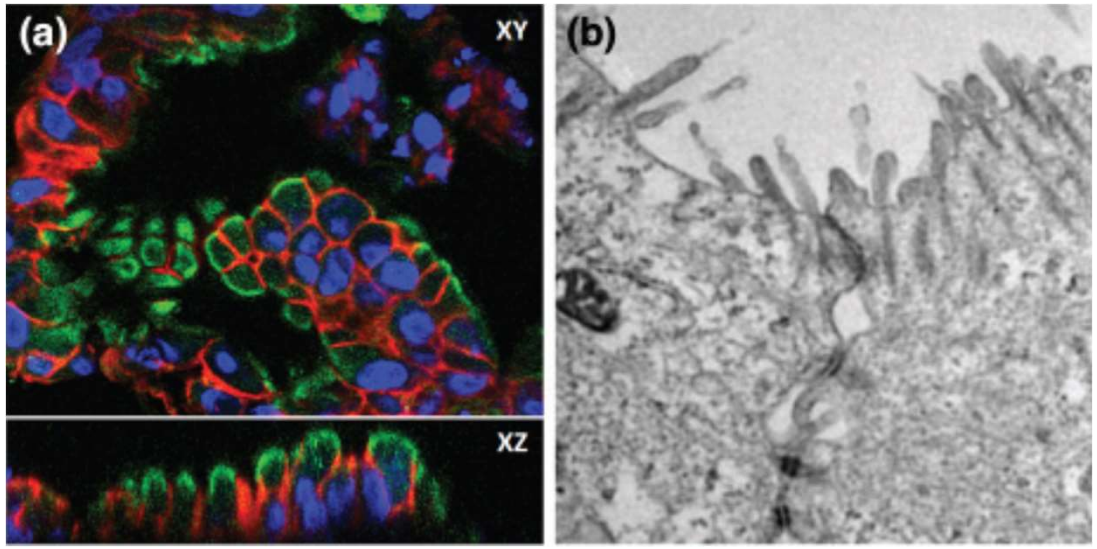
Replication of human noroviruses in stem cell–derived human enteroids

Khalil Ettayebi,[1*] Sue E. Crawford,[1*] Kosuke Murakami,[1*] James R. Broughman,[1] Umesh Karandikar,[1] Victoria R. Tenge,[1] Frederick H. Neill,[1] Sarah E. Blutt,[1] Xi-Lei Zeng,[1] Lin Qu,[1] Baijun Kou,[1] Antone R. Opekun,[2,3,4] Douglas Burrin,[3,4] David Y. Graham,[1,2,5] Sasirekha Ramani,[1] Robert L. Atmar,[1,2] Mary K. Estes[1,2†]

(Ettayebi et al. 2016)

The Enteroid Strikes Back

- HIE Recipe:
  - Isolate stem cells from human intestinal crypts
  - Provide growth factors and nutrients to develop into differentiated “mini guts”



(Kovbasnjuk et al. 2013)

The Enteroid Strikes Back

- HIE Recipe:
  - Isolate stem cells from human intestinal crypts
  - Provide growth factors and nutrients to develop into differentiated “mini guts”
  - Behave like intestine, multiple intestinal epithelial cell types: **enterocytes**, goblet, enteroendocrine, Paneth cells
    - Can be 3D or grown as monolayer

(Ettayebi et al. 2016; Kovbasnjuk et al. 2013)

Enteroids for Inactivation

- Aged green tea; heat; chlorine; ethanol
- Real value in comparison to surrogates

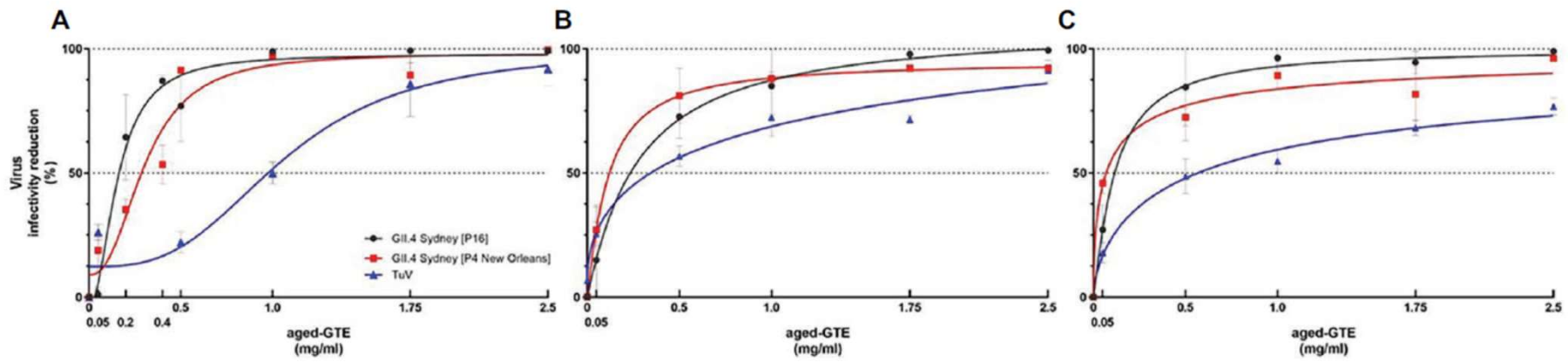
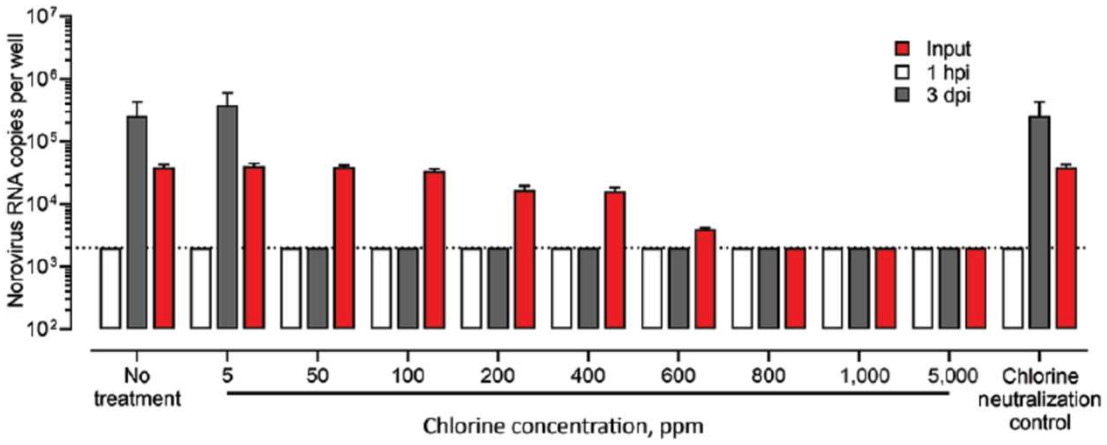


FIGURE 4 | Effective concentration (EC_{50}) of aged-GTE that inhibits human norovirus and Tulane virus replication. Viruses were exposed to aged-GTE for 1 h at 37°C **(A)**, 21°C **(B)**, and 7°C **(C)**. A nonlinear regression (curve fit) function on data sets of three experiments with three technical replicates for each treatment, time point, and virus were used to obtain EC_{50} values. The data is based on genomic copies for human norovirus and on $TCID_{50}$ for Tulane virus.

(Ettayebi et al. 2016; Constantini et al. 2018; Randazzo et al. 2020)

Enteroids for Inactivation

- Aged green tea; heat; chlorine; ethanol
- Real value in comparison to surrogates
- 60^{o}C for 15 min; 50 ppm chlorine for 1 min



(Ettayebi et al. 2016; Constantini et al. 2018; Randazzo et al. 2020)

Enteroids for Inactivation

- Aged green tea; heat; chlorine; ethanol
- Real value in comparison to surrogates
- 60^{o}C for 15 min; 50 ppm chlorine for 1 min
- Still utilize RT-qPCR
- Not much sensitivity (~2 log reduction)
- Great model for antivirals/therapeutics

(Ettayebi et al. 2016; Constantini et al. 2018; Randazzo et al. 2020)

HIE Model: Ready for Our Purposes?

- Major breakthrough, both models game-changers
- Replicated in multiple labs
- Great for studying HuNoV biology/infection mechanisms
- Not the highest production
  - Not high enough to provide ideal stock sensitivity for testing inactivation agents
- **Bottom line for applied purposes**: Stay tuned

Noroviruses Can Potentially have Good Effects?

- Some evidence in germ-free and antibiotic-treated mice with MNV

LETTER

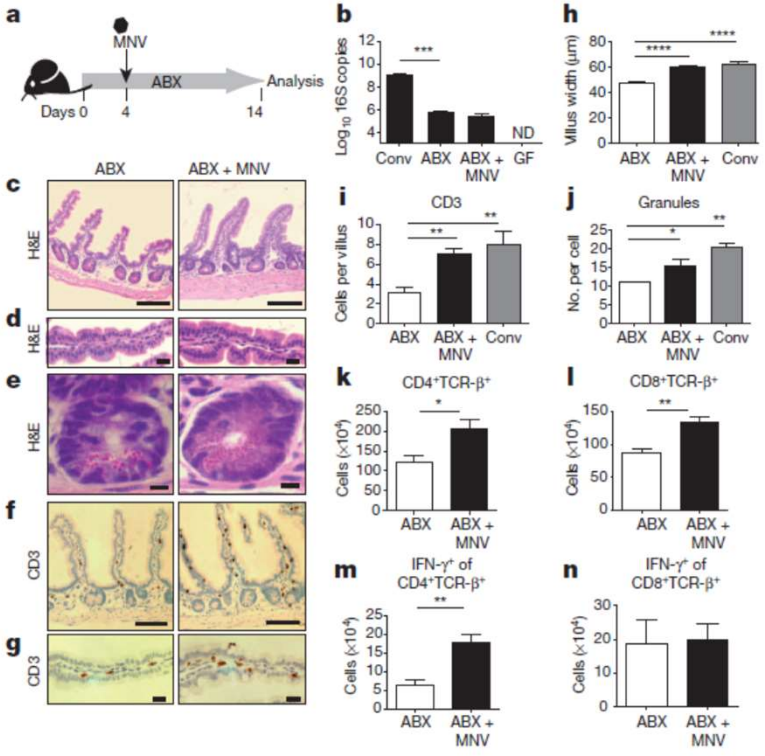
doi:10.1038/nature13960

An enteric virus can replace the beneficial function of commensal bacteria

Elisabeth Kernbauer[1,2], Yi Ding[3,4] & Ken Cadwell[1,2]

Noroviruses Can Potentially have Good Effects?

- Improved intestinal morphology and mucosal immune function



(Kernbauer et al. 2014)

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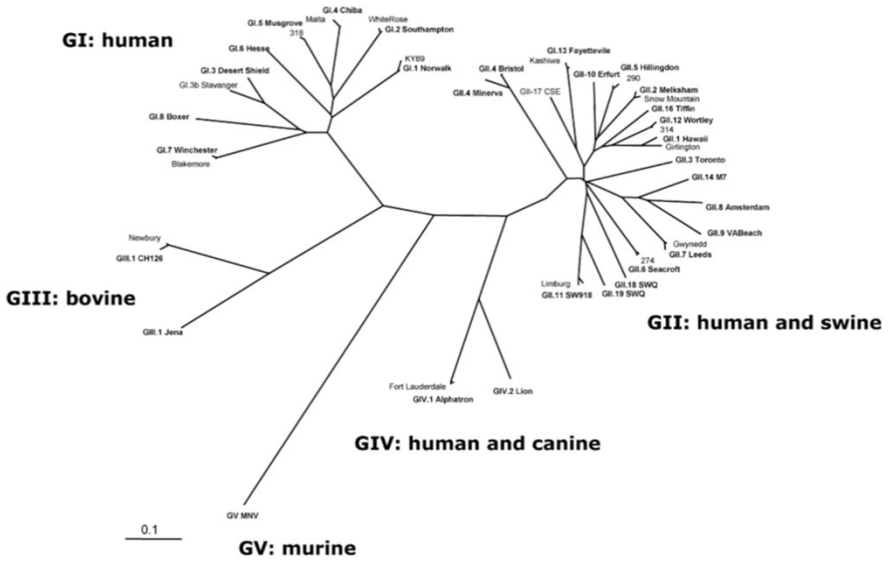
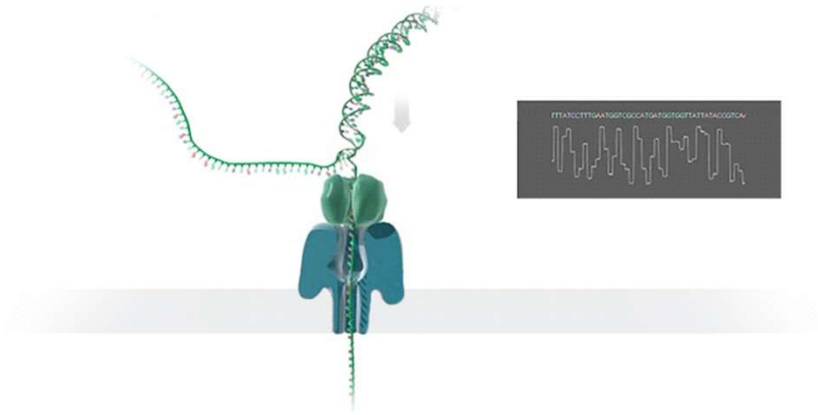


(Vinje 2014; Bull et al. 2010; Patel 2009)

In-Field, Point-of-Service Detection: WGS

- Whole genome sequencing
- Nanopore sequencing: MINion
- How it works
- Size of graphing calculator, 90g
- Powered by USB to computer
- Real-time reads to laptop
- Reads up to 60 kb (NoV= 7.5 kb)
- Can read straight RNA
- $1,000 to get started



(Hoenen et al. 2016; Wang et al. 2015; Oxford Nanopore Technologies)

In-Field, Point-of-Service Detection: WGS

- Whole genome sequencing
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(Hoenen et al. 2016; Wang et al. 2015; Ibtimes.com)

In-Field, Point-of-Service Detection: WGS

- Has been used for other viruses
  - Influenza
  - **Ebola**
  - Poxviruses
  - Lambda bacteriophage
- Usually requires amplification
- Not yet used for foodborne viruses
- Challenges: base calling accuracy, data analysis

(Hoenen et al. 2016; Wang et al. 2015; The Atlantic)

In-Field, Point-of-Service Detection: WGS

- Able to detect GII norovirus from fecal samples using 3rd generation sequencing technology (MinION and PacBio Sequel)

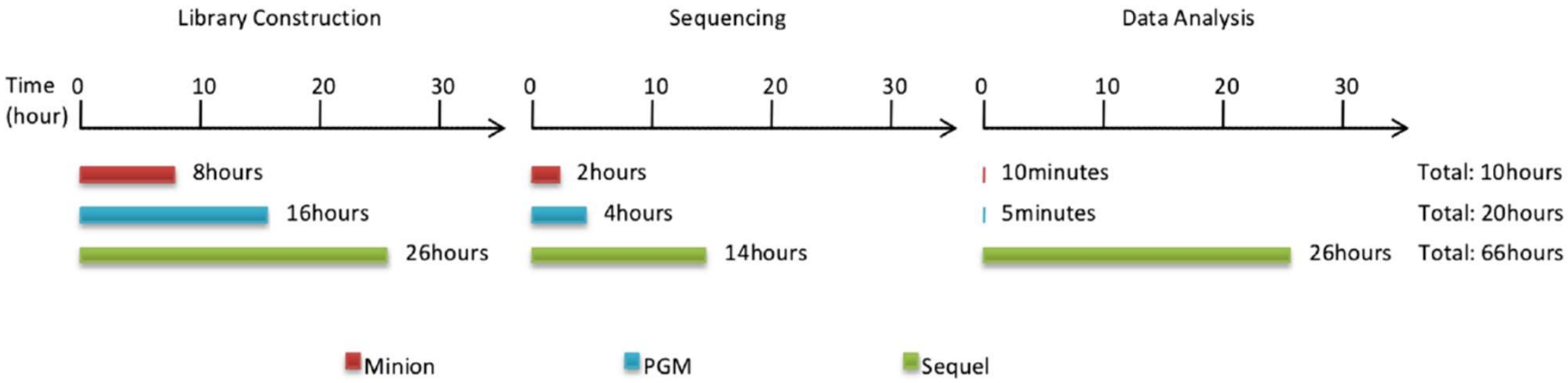
Comparison of third-generation sequencing approaches to identify viral pathogens under public health emergency conditions

Yang Li[1] · Xiao-zhou He[1] · Ming-hui Li[2] · Bo Li[3] · Meng-jie Yang[1] · Yao Xie[2] · Yi Zhang[1] · Xue-jun Ma[1,4]

(Li et al. 2020)

In-Field, Point-of-Service Detection: WGS

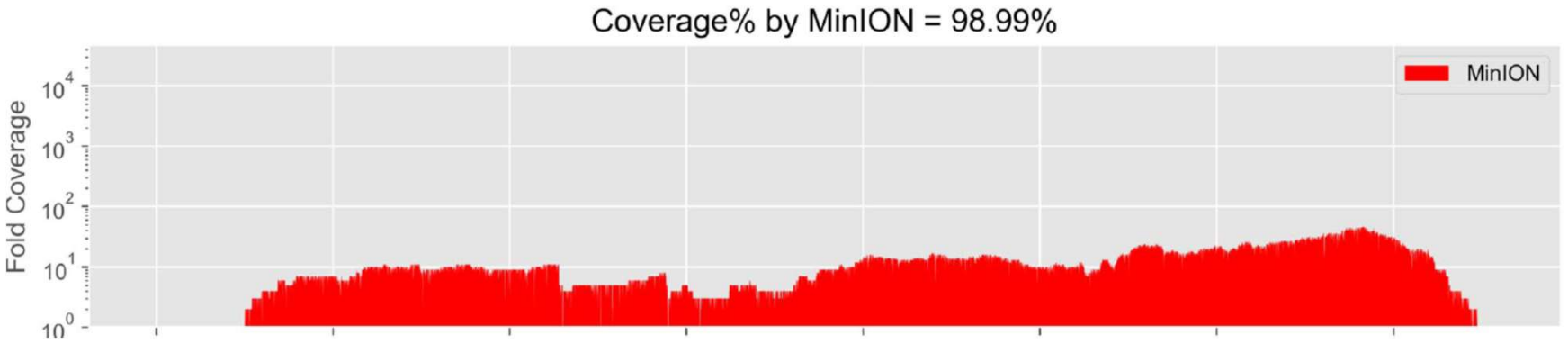
- Able to detect GII norovirus from fecal samples
- Comparatively quicker sample-to-result (~10 hours)



(Li et al. 2020)

In-Field, Point-of-Service Detection: WGS

- Able to detect GII norovirus from fecal samples
- Comparatively quicker sample-to-result (~10 hours)
- Not amazing depth, but enough coverage to be valuable



(Li et al. 2020)

In-Field, Point-of-Service Detection: WGS

- Able to detect GII norovirus from fecal samples
- Comparatively quicker sample-to-result (~10 hours)
- Not amazing depth, but enough coverage to be valuable
- Only applicable for clinical samples at this time
- Implications for identification of outbreaks

(Li et al. 2020)

Conclusions

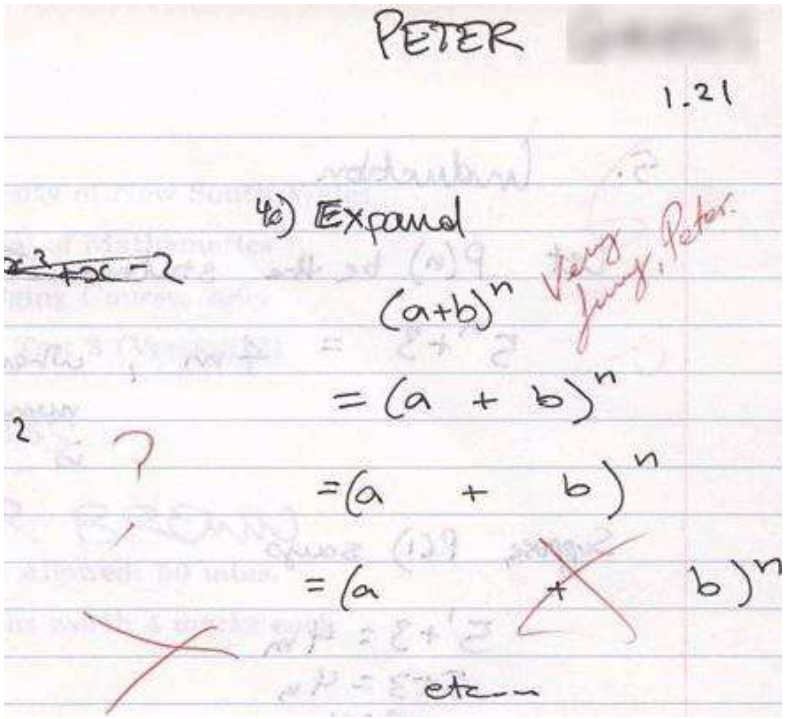
- Norovirus imposes huge burden globally and in US
- *Seemingly* small and simple structurally
- Many challenges
  - Infectious dose, diversity, multiple transmission routes, stability, requirement for concentration, asymptomatic shedding
  - Big recent developments: cell culture models; vaccine development, potential beneficial effects, 3rd-generation sequencing
- **Major take-aways from this presentation**:
  - Noroviruses present MANY challenges, but a lot of discoveries are likely to occur in the next 5 years
  - Even with breakthroughs in fundamental understanding, challenges will still be difficult in our field

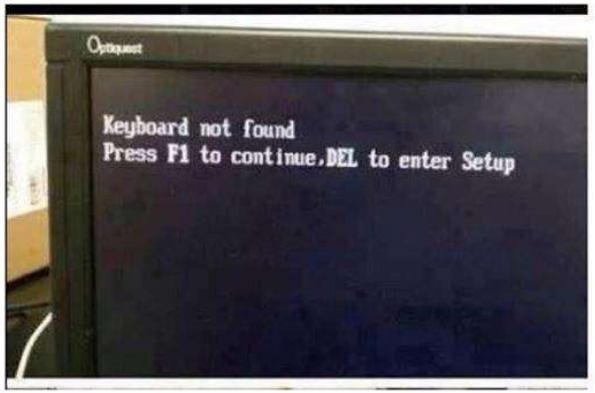
Acknowledgements

- Everyone attending!
- NEHA
- Dr. Clyde Manuel
- Laura Wildey
- Terryn Laird
- UMass Department of Food Science

- **Our lab:**
- Anand Soorneedi
- Cassie Suther
- Christina Wormald
- Cindy Kane
- Hao-Yuan Hsu
- Minji Kim
- Pragathi Kamarasu
- Louisa Bachman
- Melina Demokritou
- Nicholas Holt
- Sloane Stoufer

Questions?


PETER
1.21
b) Expand
$(a+b)^n$
Very funny, Peter.
$=(a+b)^n$
$=(a+b)^n$
$=(a+b)^n$
etc.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