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Serological surveillance of noroviruses in a community-based prospective cohort: a study protocol

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ABSTRACT

Introduction Noroviruses are the leading cause of viral acute gastroenteritis affecting all age groups. Since 2014, the previous rarely reported GII.P17-GII.17 and recombinant GII.P16-GII.2 norovirus emerged, replacing GII.4 predominant genotype, causing increased outbreaks in China and other countries. Meanwhile, GII.4/2012 Sydney strain has re-emerged as the dominant variant in many places in 2015–2018. The role of herd immunity as the driving force during these new emerging or re-emerging noroviruses is poorly defined. Serological surveillance studies on community-based prospective cohort on norovirus are highly needed.

Methods and analyses This study will include 1000 out of 9798 participants aged 18 years and above from Caofeidian district, Tangshan city, northern China. Baseline data on sociodemographic characteristics and blood samples were collected in 2013–2014. Blood collection will be replicated annually throughout the cohort until 2023. Saliva samples were also collected in 2016. The seroprevalence and seroincidence of blockade antibodies against norovirus genotypes of GII.P17-GII.17, GII.P16-GII.2, the re-emerged GII.4/2012 and potential novel pandemic variants will be evaluated by ELISA. Associations between genotype blockade antibodies and sociodemographic factors and human histo-blood group antigens will be evaluated using univariate and multivariate analysis. The dynamics of herd immunity duration will be estimated in this longitudinal surveillance.

Ethics and dissemination The study has been approved by the Ethical Committees of the Staff Hospital of Jidong oil-field of China National Petroleum Corporation. This study will provide insight into the seroprevalence and seroincidence of noroviruses, and their relationships with sociodemographic characteristics and genetic susceptibility. It will also explain herd immunity of the emerged and re-emerged genotypes or variants. The study will further enable an understanding of the mechanism driving the replacement of norovirus genotypes. Research findings will be disseminated in peer-reviewed journals and at scientific meetings.

Strengths and limitations of this study

- Jidong cohort is a community-based, large-scale and prospective cohort study with a 10-year follow-up designed to establish a health-related database and biobank.
- The cohort will provide an optimal longitudinal population to elucidate the mechanism driving the substitution of norovirus genotypes, as time covers the emergence or re-emergence of GII.P17-GII.17, GII.P16-GII.2 and GII.4/2012.
- This study is first to illuminate immune-dynamics and duration of herd immunity in pre, during and post epidemics of the emerged and re-emerged genotypes or variants.
- Due to a single-centre study and exclusion of the population under 18 years old, it may result in under-representation.
- Analysis of seroprevalence data will be complicated by heterotypic or cross-genotype immunty and lack of molecular surveillance data to identify which noroviruse genotypes and variants are circulating within the study location.

INTRODUCTION

Noroviruses (NoVs) remain the leading viral cause of acute gastroenteritis (AGE) worldwide, causing approximately one-fifth of global AGE.[1] In developed countries, NoVs result in approximately 900 000 clinical visits among children per year and 64 000 episodes of diarrhoea hospitalisation. They account for 200 000 deaths of children under 5 in developing countries.[2] The incidence of NoV-associated diseases is highest in children under 5 years old, and severe outcomes, such as death and hospitalisation, are most common among both young children and the elderly.[3] Globally, NoVs lead to approximately 4.2 billion dollars in direct healthcare costs and 60.3 billion dollars in societal costs annually.[4] Currently, NoV outbreaks, epidemics and pandemics posed a significant public health threat globally.[5]

Belonging to the Caliciviridae family, NoVs are single-stranded, positive-sense RNA genome, with an error-prone RNA polymerase.[6] The genome contains three open reading frames (ORFs). ORF1 encodes non-structural proteins, including an

RNA-dependent RNA polymerase (RdRp), while ORF2 and ORF3 encode the major (VP1), and minor (VP2) structural protein, respectively. VP1 is composed of the shell (S domain) and the surface protruding domain (P domain), which contains both the antigenic and histo-blood group antigen (HBGA) binding sites.[7 8] Based on the RdRp and VP1 gene sequences, NoVs are divided into 10 genogroups (G) of which GI, GII and GIV have been reported in humans.[9 10] Genogroup I and GII are further subdivided into at least 9 and 27 genotypes, respectively, with GII.4 accounting for the majority (70%–80%) of global NoV outbreaks for almost two decades.[9 11]

However, in the winters of 2014/2015 and 2015/2016, NoV epidemic seasons, the previously rare GII.P17-GII.17 NoV strains emerged and caused sudden increase of outbreaks or epidemics in East Asia and many other countries.[12–14] Moreover, from 2016 to 2017 epidemic season, recombinant GII.P16-GII.2 emerged, also resulting in an increase of NoV outbreaks in various countries.[15–17] Meanwhile, in 2015–2018 seasons, GII.4/2012 Sydney variants (GII.Pe-GII.4 and GII.P16-GII.4) re-emerged and were circulating in many countries, including USA, France and China.[18–20] The resurgence of GII.4/2012 made it more important and urgent to elucidate the evolutional and epidemiological mechanisms of NoVs. The mechanism driving NoV evolution has always been an important issue, but lacks clarification. Factors, such as antigenic shift under selective pressure of herd immunity, polymerase fidelity, recombination, host susceptibility and lack of herd immunity, may contribute to NoV evolution.[12 19 21 22]

This protocol will focus on the role of herd immunity as a driving force in NoV substitution. Herd immunity, protection of a population to a specific pathogen, is considered playing a critical role in altering new NoV variants.[23 24] With the emergence of these strains replacing the previously dominant strains, the mechanism of herd immunity against predominance of emerged or re-emerged NoV strains needs to be elucidated. Serological data are useful to speculate dynamic changes in susceptibility and immune profile.[25] Quantifying antibody responses and dynamics against different NoV strains have significant implications in seroincidence estimation.[26] Thus, longitudinal serological surveillance studies on community-based prospective cohorts of NoVs are urgently needed. Additionally, human HBGAs are viral attachment factors crucial for susceptibility to NoV-associated diarrhoea and exhibit varying affinity to NoV genotypes.[27 28] The association between HBGA status, NoV infections and detection of blockade antibodies are of great essence to evaluate the risk in a specific population.

AIMS AND OBJECTIVES

The aim of this study is to perform a serological surveillance of NoV in a community-based prospective cohort. This will provide thorough insight on seroprevalence and seroincidence of different NoV genotypes, and the relationships with sociodemographic characteristics and genetic susceptibility. Herd immunity elucidation will further help in understanding the mechanism for replacing specific NoV strains. Understanding herd immunity and its duration will also provide important data for vaccine development against NoVs.

Study objectives

1. To evaluate the seroprevalence and seroincidence of GI and GII strains in a community-based population.
2. To determine associations between blockade antibodies against different NoV genotypes and sociodemographic characteristics and genetic susceptibility.
3. To elucidate the spectrum and dynamics of blockade antibodies and of immunity for pre, during, and post epidemic against GII.P17-GII.17, GII.P16-GII.2 and GII.4/2012 and novel pandemic strains.
4. To estimate the duration of herd immunity of GII.P17-GII.17, GII.P16-GII.2 and GII.4/2012.

METHODS AND ANALYSIS

Study setting and population

This study cohort is conducted at Jidong Community, situated in Caofeidian district, Tangshan city, Hebei Province, northern China. Caofeidian district has an area of 1944 km^2 and a population of 268.7 thousand people (according to 2012 China census), locates in the south of the Tangshan city and close to the Bohai sea. Tangshan is a large, modern industrialised city seated in the central section of the circum-Bohai region, where it is adjacent to two metropolitan cities: Beijing and Tianjin.

All participants involved in the baseline investigation were 18 years old and above. This cohort study comprised of 9798 Caofeidian participants. Our study recruited 1000 participants using stratified random sampling by gender and age.

Study overview and design

This project will be composed of three components, as follows:

1. Collection of baseline information and biological samples.
2. Laboratory methods.
3. Statistical and modelling analysis.

Collection of baseline information and biological samples (component one)

Baseline investigation on sociodemographic characteristics (eg, age, gender, dietary intake, occupational exposure and medical history) and blood samples were collected from July 2013 to August 2014. Subsequent blood samples of participants involved in the cohort will be collected annually up to 2023. Paired saliva samples were also collected from participants in 2016 and stored at a temperature of −80°C.

Table 1 The list of genotypes for construction of norovirus (NoV) P proteins

NoV P proteins	Strains and GenBank accession
GI.2	KF306212 Norovirus Hu/GI.2/Jingzhou/2013401/CHN
GI.3	MT860989 Norovirus Hu/GI.3/Guigang 01/2018/CHN
GI.9	MT862216 Norovirus Hu/GI.9/Guangzhou/2016/CHN
GII.2	KY485122 Norovirus Hu/GII.2/Guangdong/CHN/2016
GII.4	AFV08795 Norovirus Hu/Sydney/NSW0514/2012 Novel pandemic GII.4 variant*
GII.6	MT861044 Norovirus Hu/GII.6/Jinshan 06/2018
GII.17	KU557815 Norovirus Hu/GII.17/16–1669/GD-DG/2014

*P protein of any novel potential pandemic GII.4 during 2013–2023 will be expressed.

Laboratory methods (component two)

Preparation of NoV P proteins

Multiple NoV P proteins listed in table 1, representing different GI and GII strains, will be expressed as described previously.[29–32]

Detection of HBGAs in saliva

The HBGA phenotypes of A, B, H and lewis a (Le^a), Le^b, Le^x and Le^y antigens in the paired saliva samples will be identified as described previously through ELISA using corresponding monoclonal antibodies.[29–32] Confirmed positive and negative saliva samples will be used as controls.

Detection for NoV blockade antibodies in sera

Blockade IgG to HBGA–NoV binding will be determined by ELISA as previously described.[29–32] The profile of blocking antibody titres will be used to calculate the seroprevalence and seroincidence for NoV and the variation in titres will be crucial to infer the duration of natural immunity.

Statistical and modeling analysis (component three)

Study analysis will be conducted as follows:

1. NoV GI and GII seroprevalence and seroincidence will be computed in line with specific blockade antibodies.
2. Univariate analysis will be performed using IBM SPSS 22.0 software (SPSS, Chicago, Illinois, USA) to describe the associations between the positive rate of individual NoV genotypes and baseline sociodemographic data of study participants (such as age, gender, occupational exposure, etc.), ABO blood groups, Lewis status and secretor status. Associated risk factors will be screened by performing Univariate analysis followed by multivariate analysis.
3. The duration of herd immunity will be roughly estimated based on the changes in the blockade antibody titres using a mathematical model in consideration of antibody dynamics at multiple timescales.

ETHICS AND DISSEMINATION

This research follow the guidelines of the Helsinki Declaration.[33] The study has been approved by the Ethical Committees of the Staff Hospital of Jidong oil-field of China National Petroleum Corporation. All study participants consented prior to enrollment. The research will provide detailed information about NoV seroprevalence, seroincidence, genetic vulnerability and sociodemographic risk factors. This work will also explain the herd immunity of GII.P17-GII.17, GII.P16-GII.2 and GII.4/2012. It would allow for a comprehensive understanding of the mechanism of replacement of NoV genotypes. Study results will be published in peer-reviewed journals and disseminated in the fields of infectious diseases, vaccines, immunity and monitoring at scientific meetings.

DISCUSSION

Strengths

We will conduct a 10-year longitudinal serological cohort with regards to norovirus based on a natural population in Jidong community. The seroincidence and seroprevalence will be estimated by detecting blockade antibodies against GI and GII NoV strains. Seropositive risk factors can be identified and correlations between genotype blockade antibodies, sociodemographic characteristics and genetic susceptibility to NoV infection can be illustrated.

The cohort began in 2013–2014 and will end in 2023, during which GII.P17-GII.17 and GII.P16-GII.2 replaced GII.4/2012 to become predominant. Of note, in 2015–2018 seasons, GII.4/2012 (GII.Pe-GII.4 and GII.P16-GII.4) re-emerged as the dominant variants circulating in many countries including China.[20] Tohma *et al* suggested GII.P16 polymerase could have a positive impact on the transmissibility of the re-emerging GII.4/2012 strain. Furthermore, they proposed that since the non-GII.4 strains could not evolve antigenically, its genotype would prevail for only a short period before shifting to another genotype.[21] Our protocol will provide an optimal longitudinal population to probe mechanism of molecular evolution of emerging NoV variants and the association between the seroepidemiology and alteration of genotypes.

Despite several studies on the duration of herd immunity to NoV,[34–36] it remains poorly understood and hence urgent needs for clarification. This 10-year longitudinal cohort will explore the spectrum and the persistence of blockade antibodies against GII.P17-GII.17, GII.P16-GII.2 and GII.4/2012 in pre, during, and post epidemics. Thus, duration of herd immunity to NoV can be estimated, a significant finding for vaccine development.

Limitations

The cohort exempts persons under 18 years, especially excludes children under 5 years who are more significantly vulnerable.[3 37] Therefore, the community population is not adequately represented.

Analysis of seroprevalence data will be complicated by the lack of molecular surveillance data to identify which NoV genotypes and variants are circulating within the study location. Meanwhile, heterotypic immunity is likely to be generated on repeated infection of adults and has been also confirmed in many other studies.[38–40] Using blockade antibodies will minimise cross-reactivity between stains to maximal extent, while it will also complicate inferences from seroprevalence data.

Study site selection was limited to the Jidong community centre with the regional disparity that may affect the generalisation of our results. Another limitation is the loss to follow-up, which is inevitable in all cohort studies. This bias will be reduced by participants' follow-up via face-to-face interviews once annually. The follow-up will be a routine medical examination up to 31 December 2023, or up to the occurrence of emigration or death.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study has been approved by Ethical Committees of the Staff Hospital of Jidong oil-field of Chinese National Petroleum.

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REFERENCES

1 van Beek J, de Graaf M, Al-Hello H, *et al*. Molecular surveillance of norovirus, 2005-16: an epidemiological analysis of data collected from the NoroNet network. *Lancet Infect Dis* 2018;18:545–53.
2 Patel MM, Widdowson M-A, Glass RI, *et al*. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis* 2008;14:1224–31.
3 Lopman BA, Steele D, Kirkwood CD, *et al*. The vast and varied global burden of norovirus: prospects for prevention and control. *PLoS Med* 2016;13:e1001999.
4 Bartsch SM, Lopman BA, Ozawa S, *et al*. Global economic burden of norovirus gastroenteritis. *PLoS One* 2016;11:e0151219.
5 Ahmed SM, Hall AJ, Robinson AE, *et al*. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *Lancet Infect Dis* 2014;14:725–30.
6 Lindesmith LC, Brewer-Jensen PD, Mallory ML, *et al*. Human norovirus epitope D plasticity allows escape from antibody immunity without loss of capacity for binding cellular ligands. *J Virol* 2019;93. doi:10.1128/JVI.01813-18. [Epub ahead of print: 15 Jan 2019].
7 Vega E, Barclay L, Gregoricus N, *et al*. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. *J Clin Microbiol* 2014;52:147–55.
8 Vega E, Barclay L, Gregoricus N, *et al*. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. *Emerg Infect Dis* 2011;17:1389–95.
9 Chhabra P, de Graaf M, Parra GI, *et al*. Updated classification of norovirus genogroups and genotypes. *J Gen Virol* 2019;100:1393–406.
10 Ford-Siltz LA, Mullis L, Sanad YM, *et al*. Genomics analyses of GIV and GVI noroviruses reveal the distinct clustering of human and animal viruses. *Viruses* 2019;11. doi:10.3390/v11030204. [Epub ahead of print: 01 Mar 2019].
11 van Beek J, Ambert-Balay K, Botteldoorn N, *et al*. Indications for worldwide increased norovirus activity associated with emergence of a new variant of genotype II.4, late 2012. *Euro Surveill* 2013;18:8–9.
12 Chan MCW, Lee N, Hung T-N, *et al*. Rapid emergence and predominance of a broadly recognizing and fast-evolving norovirus GII.17 variant in late 2014. *Nat Commun* 2015;6:10061.
13 Fu J, Ai J, Jin M, *et al*. Emergence of a new GII.17 norovirus variant in patients with acute gastroenteritis in Jiangsu, China, September 2014 to March 2015. *Euro Surveill* 2015;20:21157.
14 Matsushima Y, Ishikawa M, Shimizu T, *et al*. Genetic analyses of GII.17 norovirus strains in diarrheal disease outbreaks from December 2014 to March 2015 in Japan reveal a novel polymerase sequence and amino acid substitutions in the capsid region. *Euro Surveill* 2015;20. doi:10.2807/1560-7917.ES2015.20.26.21173. [Epub ahead of print: 02 Jul 2015].
15 Niendorf S, Jacobsen S, Faber M, *et al*. Steep rise in norovirus cases and emergence of a new recombinant strain GII.P16-GII.2, Germany, winter 2016. *Euro Surveill* 2017;22. doi:10.2807/1560-7917.ES.2017.22.4.30447. [Epub ahead of print: 26 Jan 2017].
16 Ao Y, Wang J, Ling H, *et al*. Norovirus GII.P16/GII.2-Associated gastroenteritis, China, 2016. *Emerg Infect Dis* 2017;23:1172–5.
17 Thongprachum A, Okitsu S, Khamrin P, *et al*. Emergence of norovirus GII.2 and its novel recombination during the gastroenteritis outbreak in Japanese children in mid-2016. *Infect Genet Evol* 2017;51:86–8.
18 Bidalot M, Théry L, Kaplon J, *et al*. Emergence of new recombinant noroviruses GII.p16-GII.4 and GII.p16-GII.2, France, winter 2016 to 2017. *Euro Surveill* 2017;22. doi:10.2807/1560-7917.ES.2017.22.15.30508. [Epub ahead of print: 13 Apr 2017].
19 Ruis C, Roy S, Brown JR, *et al*. The emerging GII.P16-GII.4 Sydney 2012 norovirus lineage is circulating worldwide, arose by late-2014 and contains polymerase changes that may increase virus transmission. *PLoS One* 2017;12:e0179572.
20 Xue L, Cai W, Gao J, *et al*. The resurgence of the norovirus GII.4 variant associated with sporadic gastroenteritis in the post-GII.17 period in South China, 2015 to 2017. *BMC Infect Dis* 2019;19.
21 Tohma K, Lepore CJ, Ford-Siltz LA, *et al*. Phylogenetic analyses suggest that factors other than the capsid protein play a role in the epidemic potential of GII.2 norovirus. *mSphere* 2017;2. doi:10.1128/mSphereDirect.00187-17. [Epub ahead of print: 17 May 2017].
22 Lindesmith LC, Donaldson EF, Lobue AD, *et al*. Mechanisms of GII.4 norovirus persistence in human populations. *PLoS Med* 2008;5:e31.
23 Cannon JL, Lindesmith LC, Donaldson EF, *et al*. Herd immunity to GII.4 noroviruses is supported by outbreak patient sera. *J Virol* 2009;83:5363–74.
24 Metcalf CJE, Ferrari M, Graham AL, *et al*. Understanding herd immunity. *Trends Immunol* 2015;36:753–5.
25 Metcalf CJE, Farrar J, Cutts FT, *et al*. Use of serological surveys to generate key insights into the changing global landscape of infectious disease. *Lancet* 2016;388:728–30.
26 Kucharski AJ, Lessler J, Cummings DAT, *et al*. Timescales of influenza A/H3N2 antibody dynamics. *PLoS Biol* 2018;16:e2004974.
27 Yang Y, Xia M, Tan M, *et al*. Genetic and phenotypic characterization of GII-4 noroviruses that circulated during 1987 to 2008. *J Virol* 2010;84:9595–607.

28 Hutson AM, Atmar RL, Marcus DM, *et al*. Norwalk virus-like particle hemagglutination by binding to H histo-blood group antigens. *J Virol* 2003;77:405–15.
29 Dai Y-C, Zhang X-F, Xia M, *et al*. Antigenic relatedness of norovirus GII.4 variants determined by human challenge sera. *PLoS One* 2015;10:e0124945.
30 Dai Y-C, Xia M, Huang Q, *et al*. Characterization of antigenic relatedness between GII.4 and GII.17 noroviruses by use of serum samples from Norovirus-Infected patients. *J Clin Microbiol* 2017;55:3366–73.
31 Xie D, Chen J, Yu J, *et al*. Characterization of antigenic relatedness among Gi norovirus genotypes using serum samples from Norovirus-Infected patients and mouse sera. *Front Microbiol* 2020;11:607723.
32 Zhang XF, Chen JR, Song CL, *et al*. Characterization of a hospital-based gastroenteritis outbreak caused by GII.6 norovirus in Jinshan, China. *Epidemiol Infect* 2020;148.
33 World Medical Association. World Medical association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;310:2191–4.
34 Simmons K, Gambhir M, Leon J, *et al*. Duration of immunity to norovirus gastroenteritis. *Emerg Infect Dis* 2013;19:1260–7.
35 Parrino TA, Schreiber DS, Trier JS, *et al*. Clinical immunity in acute gastroenteritis caused by Norwalk agent. *N Engl J Med* 1977;297:86–9.
36 Sakon N, Yamazaki K, Nakata K, *et al*. Impact of genotype-specific herd immunity on the circulatory dynamism of norovirus: a 10-year longitudinal study of viral acute gastroenteritis. *J Infect Dis* 2015;211:879–88.
37 Bierhoff M, Arvelo W, Estevez A, *et al*. Incidence and clinical profile of norovirus disease in Guatemala, 2008-2013. *Clin Infect Dis* 2018;67:430–6.
38 Lindesmith LC, McDaniel JR, Changela A, *et al*. Sera antibody repertoire analyses reveal mechanisms of broad and pandemic strain neutralizing responses after human norovirus vaccination. *Immunity* 2019;50:1530–41.
39 Lindesmith LC, Ferris MT, Mullan CW, *et al*. Broad blockade antibody responses in human volunteers after immunization with a multivalent norovirus VLP candidate vaccine: immunological analyses from a phase I clinical trial. *PLoS Med* 2015;12:e1001807.
40 Lindesmith LC, Beltramello M, Donaldson EF, *et al*. Immunogenetic mechanisms driving norovirus GII.4 antigenic variation. *PLoS Pathog* 2012;8:e1002705.