Chapter

Norovirus: Clinical Findings and Pharmaceutical Developments

Ying-Fei Yang and Chung-Min Liao

Abstract

Norovirus (NoV) is one of the most ubiquitous factors contributing to acute gastroenteritis that causes widespread outbreaks in travel industry, military, or healthcare facilities. NoV could lead to serious symptoms and result in severe societal costs worldwide. Surprisingly, there has been no available licensed vaccines, albeit there are ongoing pre-clinical or clinical trials of several candidate vaccines. Development of effective universal vaccines has been found difficult clinically due to the frequent point mutations and a lack of robust animal model and cell culture system. Preclinical studies showed that vaccines with virus-like particles (VLPs) have high immunogenicity and efficacies and were demonstrated to be protective and safe. Recent *in vitro* research also suggests that human intestinal enteroids can enhance our understanding of protection mechanism and give guidance for vaccine development. Overall, this chapter will give a comprehensive review of the current challenge and progress of clinical findings, efficacy/safety of the developing vaccines, and antiviral drug developments for NoV in clinical trials or preclinical investigations.

Keywords: norovirus, gastroenteritis, vaccine, immunogenicity, clinical findings

1. Introduction

Norovirus (NoV) has been the leading cause contributing to acute gastroenteritis worldwide [1]. The Product Development for Vaccines Advisory Committee of the World Health Organization (WHO) also recognized NoV as a priority disease for vaccine development [1]. It was estimated that the global burden of NoV disease was more than 677 million cases and 213515 deaths annually [1]. Surveillance studies in the United States suggested that NoV leads to ~20% of acute gastroenteritis in children under 5 years old [2–4]. It was also demonstrated that rate of NoV gastroenteritis in this group of age exceeds that of rotavirus gastroenteritis [2–4]. Particularly high rates of mortality and illness requiring medical care present at age > 65 years and under 5 year olds, respectively [4, 5]. Sudden onset of symptoms including watery diarrhea, vomiting, abdominal cramps, malaise, and fever are commonly seen and usually more serious occur in immunocompromised or elderly individuals [1].

NoVs are small (diameter, 35–40 mm), non-enveloped, positive, and single-stranded RNA viruses belonging to the *Caliciviridae* family [1, 6]. With Norovirus being one of the genera of the family, it could be classified into seven genogroups based on the phylogenetic analysis of the virus genome and capsid sequence, where GI, GII, and GIV infect humans and GI and GII are major groups causing human infections (**Table 1**) [1]. GI and GII genogroups could be respectively subdivided into 9 and 25 genotypes based on the viral capsid protein (VP1) sequence.

Genus	Genogroup	Genotypes	Species infected
Lagovirus	GI	GI.1–9	Human
	GII	GII.1–23	Human and pig
Nebovirus	GIII	GIII.1–2	Cow and sheep
Norovirus	GIV	GIV.1–2	Human, cat, dog, and lion
Sapovirus	GV		Mouse and rat
Vesivirus	GVI	GVI.1–2	Dog
	GVII		Dog

Table 1.
Classification of the Caliciviridae *family, among which GI and GII are the most prevalent genogroups for NoV infections in human (adapted from Melhem [1] and Cortes-Penfield [33]).*

Development of NoV vaccines has been in progress for more than a decade. There has been no licensed vaccines for prevention of NoV infections [1]. Although there are several candidates that have gone through clinical trials such as the Adenovirus type 5 vaccine expressing the GI.1 capsid protein (Vaxart) and the bivalent GI and GII.4 VLP vaccine (Takeda), development of a vaccine with broad-spectrum antiviral effects against various or newly emerging viral strains are of increasingly needed [1].

2. Clinical observations

It was estimated that one in six patients with acute diarrhea has NoV as the causative pathogen [7, 8]. Nov is highly contagious and was estimated to release more than 30 million virus particles from vomiting [8–10]. The infecting dose are only 10–100 virus particles to induce related symptoms, and the attack rate is more than 50% [8–12]. Generally, the incubation period of NoV is from 24 to 48 hours. There's no prodromal illness before the onset of the NoV, and the symptoms are usually explosive [8, 12, 13]. Predominant symptoms include fever, vomiting, diarrhea and clinically lasts for 2 to 3 days [8, 14]. Vomiting and diarrhea are the most common symptoms that occurs in 70% and 90% infected patients, respectively.

In a 3-year multicentered study presenting clinical features of Norovirus in Taiwan children, fever and vomiting are found to stop in approximately 3 and 2 days, respectively [8, 14]. However, diarrhea lasts as long as around 6 days [8, 14]. Malnutrition, dehydration, and dysfunctional intestinal barrier will also worsen the illness [7, 8].

Arias et al. [15] also performed a prospective study provided evidence that symptoms such as abdominal pain, vomiting, and myalgia are common in both children and adults, while myalgia and diarrhea are more common seen in adults. There was no difference found between male and female subjects (**Figure 1**). However, fever and abdominal pain were observed to be more frequent in male than in female individuals (34.5% vs. 28.9%, $p = 0.022$; 70.8% vs. 64.4%, $p = 0.013$, respectively) (**Figure 1**) [15]. In another stratified factor, age may play as an influential role in symptoms of subjects. It was noticed that risk of diarrhea was higher in elderly (> 65 years) group (OR; 2.61; 95% CI: 1.93–3.55) (**Figure 2**) [15]. Vomiting and abdominal pain were found to be more frequent in children at age < 5 years old (**Figure 2**) [15].

It was reported that there are 3 to 10 episodes of diarrhea per day in patients with NoV gastroenteritis [16]. Moreover, NoV gastroenteritis could be a major threat to

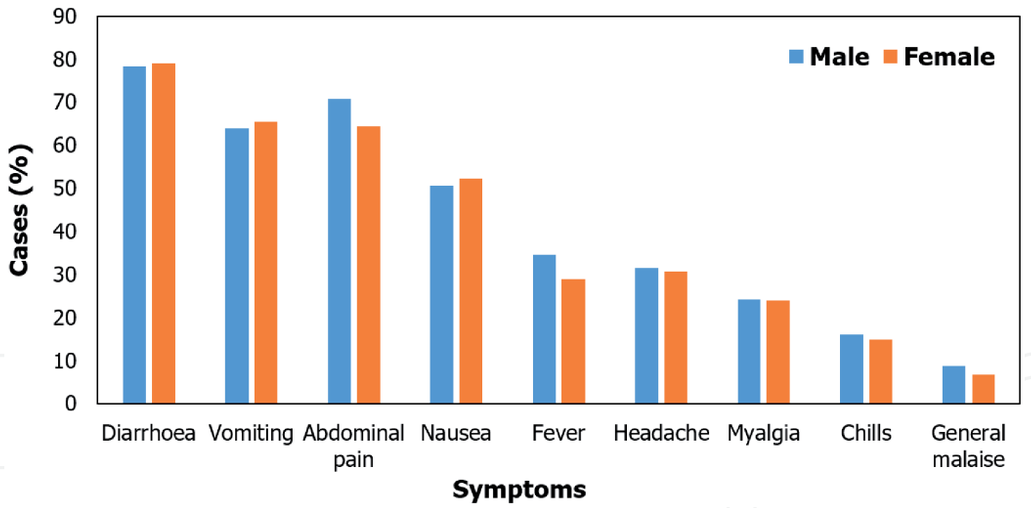

Figure 1.
Distribution of symptoms of NoV based on male and female among 1544 cases from 2004 to 2005 in Catalonia, Spain (adapted from arias et al. [15]).

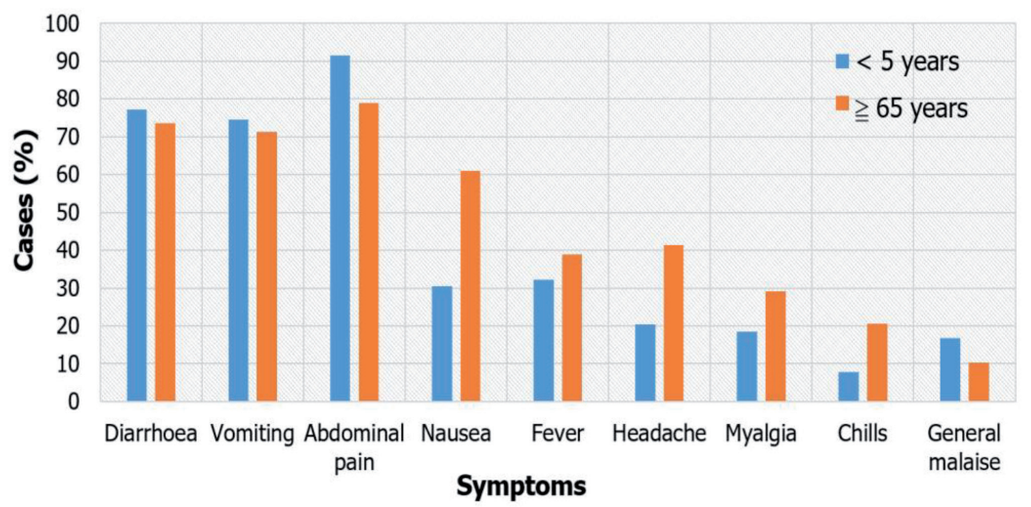

Figure 2.
NoV symptoms at two different age groups of children (< 5 years) and elderly (> 65 years) among 1544 cases from 2004 to 2005 in Catalonia, Spain (adapted from arias et al. [15]).

patients especially with hematopoietic stem cell transplant or chemotherapy [17]. It was also reported that NoV could infect individuals many times or with more than one NoV strain during the course of an outbreak [7, 8].

2.1 NoV vaccines in development

Candidate NoV vaccines in development are either in clinical trials or still in preclinical stages (**Table 2**). There have been two groups of virus-like particles (VLP)-based vaccines in development. One is a combination of GI.1 and GII.4 VLPs that is in human clinical trials, whereas the other is a mixture of GI.3 and GII.4 VLPs, which is in preclinical developments [4] (**Table 2**). In addition, there are two other groups of vaccines based on recombinant adenovirus serotype 5 vectors expressing NoV VP1. One is currently in Phase I and is based on GI.1 NoV sequence, and the other is based on GI.4 sequence and developed my Chinese Center for Disease Control and Prevention [4] (**Table 2**). Vaccines in development will be described in the following sections in this chapter.

Stage of trial	Leading Investigators	Candidate vaccine	NoV genotype	Administration route	References
Phase I	Vaxart	Recombinant Adenovirus expressing NoV VP1	GI.1	Oral	[28]
Phase IIb	Takeda	NoV VLP	GI.1, GII.4	Intramuscular	[25, 26]

Table 2.
NoV vaccines under clinical trials (adapted from Nicolas et al. [4]).

2.1.1 GI.1/Gll.4 VLP vaccines

Initially, the VLP-type NoV vaccine in human had oral administration of unadjuvanted GI.1 NoV VLPs. Results showed that there were 4-fold increments of virus-specific serum IgG level and no adverse events in 83% of the recipients [18]. In light of this finding, there's development of a VLP vaccine adjuvanted with mucoadhesin and monophosphoryl lipid A for intranasal delivery [19, 20]. The vaccine was a Phase I study with double-blind and placebo-controlled design. Results showed that it could induce NoV-specific IgG and IgA memory B cells, IgG, and IgA. No occurrence of serious adverse events [4, 19, 20].

Another randomized, double-blind, and placebo-controlled study that assessed efficacy of intranasally delivered vaccine gave subjects challenged with ~10 human-infectious doses of Norwalk virus 2 doses of vaccine/placebo [4, 21]. Results showed that a 32% absolute reduction of the risk (37% vs. 69%; $P = 0.06$) for gastroenteritis in the vaccine recipients. The result was associated with the increase of NoV-specific antibody levels including IgA and serum HBGA-blocking antibodies [4, 21]. However, there was no significant reduction of the duration of illness or symptoms. Moreover, local nasal symptoms including sneezing, itching, stuffiness, and nasal discharge were more common after second dose of vaccination in vaccine arm, regardless that there were no occurrence of serious adverse events [4, 21].

On the other hand, the prevalence of GII.4 NoV resulted in the addition of a GII.4 VLP component to GI.1 VLPs to generate bivalent vaccines [4, 22]. Previous preclinical study showed that the addition of GII.4 VLP, based on 3 different sequences of GII.4 NoV strain variants, led to induction of reactive antibodies to heterologous GI.3, GII.1, GII.3, and GIV.1 NoVs [4, 22]. Also, a randomized and placebo-controlled clinical trial of a bivalent GI.1 and consensus GII.4 VLP vaccine adjuvanted with aluminum hydroxide and monophosphoryl lipid A was delivered to subjects with a series of 2 intramuscular injections [4, 22, 23]. Developments of GI.1- and GII.4-specific serum antibody peaked at day 7 were observed after vaccination. No adverse events were distinguished. NoV-specific antibody-secreting cells, plasmablasts, and memory B cells were also evidenced. However, it was found that dose escalation did not lead to higher levels of NoV-specific antibodies. After first vaccine dose, HBGA-blocking antibodies were developed at high levels in all age groups (18–49, 50–64, and 65–85 years), whereas there was little additional increments in levels after second vaccination [4, 22, 23].

A bivalent vaccine targeting GII.4 NoV was also assessed through a randomized, double-blind, and placebo-controlled trial with 63 and 64 subjects received NoV vaccine and placebo vaccine, respectively [4, 24]. Among the participants, 56 and 53 individuals were challenged with 4400 reverse-transcription polymerase chain reaction units of a GII.4 NoV variant, which was not included in the consensus GII.4 sequence. Although there were no statistical significance in the decrease of prevalence in gastroenteritis, milder symptoms were observed after NoV challenge. No reported adverse events. No reductions of duration of NoV illness and time from challenge to onset of symptoms were discovered after vaccination [4, 24].

Another study for investigation of a bivalent GI.1/GII.4 vaccine is in Phase II clinical trials that prevention of NoV-related illness and infection after intranasally or intramuscularly administration were also demonstrated. The Phase IIb, double-blind, randomized, placebo-controlled, efficacy trial with participants from the US Navy was just performed (NCT02669121) [4, 25]. Results showed that increases of GI.1 and GII.4 HBGA-blocking antibodies in vaccines and in some placebo cases infected with GII.2 revealed the cross-activity in the immune responses to different genotypes [26].

Due to the lack of *in vitro* culture systems for human NoV, there has been no inactivated or live attenuated NoV particle vaccines developed [4]. Although there are 2 NoV candidate vaccines in human trials, the immediate development of vaccines based on NoV particles seems difficult [4].

2.1.2 Adenovirus vector-based GI.1 VP1 vaccines

It was evidenced that a recombinant serotype 5 adenovirus vector with expression of a GII.4 NoV VP1 was immunogenic in mice intranasally vaccinated [4, 27]. NoV-specific IgG and IgA in feces, respiratory mucosa, and serum were observed [4, 27]. It was also found by the same group that combination of booster vaccination using NoV VKPs and adenovirus vector could enhance immune responses [4, 27].

Another adenovirus vector-based NoV vaccine (VXA-GI.1-NN) expressing NoV major capsid protein VP1 from the GI.1 Norwalk virus that is orally administered is also in development [4, 28]. The immunogenicity and tolerability of the vaccine against H1N1 influenza expressing the same adenovirus-vectored platforms were reported [4, 29]. The VXA-GI.1-NN was announced to go through the completion of a Phase I trial (NCT02868073). Recent results showed that the vaccine had no dose-limiting toxicities and only mild/moderate adverse events presented. Immunogenicity and tolerability end points of the vaccine were also reached [30]. Serum NoV-blocking antibody levels significantly increased in recipients. No adverse events observed.

In addition, a newest phase I (NCT03721549) GI.1 study (Lot 001-09NV) sponsored by the WCCT Global was also conducted to explore its efficacy with a controlled human infection model (CHIM). Results indicated that Lot 001-09NV could be a useful challenge strain for vaccine studies aiming at the establishment of immune correlates [31]. However, development of a multivalent vaccine is in need since the vaccine did not show consistently robust immune responses to heterotypic NoV strains [4, 29].

3. Drugs in clinical development

Candidate drugs for NoV treatments are still in the process of developments although there has been intensive need for effective NoV antivirals. Most candidates are still in the early stages of preclinical trials. Human NoV culture in enteroid system are frequently applied in the preclinical research to assess safety or efficacy of candidate drugs. Although the landscape for antiviral developments is frequently changed, this chapter will give a comprehensive review of the drugs that have been approved by regulatory agencies, previously or in the process of clinical trials.

3.1 Polymerase inhibitors

3.1.1 Nucleoside analogs

Among all NoV antiviral targets, the RNA-dependent RNA polymerase (RdRp) is one of the candidates identified to be effective in inhibition of NoV activity in preclinical stages. The RdRp acts as an attractive antiviral therapy since it has the advantage

of lacking host homologs to minimize the chance of off-target adverse effects [32, 33]. The RdRp-targeting antivirals could be divided into two major classes: the nucleoside analogs (NAs) and the non-nucleoside inhibitors (NNIs). Typically, NAs inhibit RNA syntheses through mimicry of the generation of nucleoside triphosphate (NTPs) that lead to chain termination after incorporation [33, 34]. On the contrary, NNIs exert lower antiviral activity and bind allosterically to block rearrangements of conformation of the viral polymerase to form active replication complex [33, 35].

3.1.1.1 2CMC

Some of the NA-drugs were repurposed to treat human NoV since they have the mechanism of RNA synthesis inhibition for various kinds of viruses. One of the drugs is called 2CMC (2'-C-methylcytidine), which was originally served as an antiviral therapy for HCV. It also exhibited inhibition activities against flaviviruses such as West Nile virus, DENV, yellow fever virus [33, 36]. Valopicitabine, one of the oral 2CMC prodrug used against for HCV, was halted for development due to undesirable gastrointestinal effects reported [33, 37]. However, although the 2CMC was discontinued for clinical treatment, it has been widely served as a potential NoV-treating drug. It was found RNA synthesis of Norwalk replicon was dose-dependently reduced by the 2CMC [33, 38]. Murine NoV (MNV) plaque and RNA synthesis were also demonstrated to be inhibited [33, 39].

3.1.1.2 T705 (Flavipiravir)

T705 is a purine analog that showed to induce lethal mutagenesis against MNV [33, 40, 41]. It has also been approved as an influenza treatment in Japan [42]. Although it was evidenced to inhibit replication of several viruses such as Ebola virus, hantaviruses, flaviviruses, and arena viruses with *in vitro* and mouse models, it showed poor antiviral activity for MNV replication in cell cultures [33, 43].

3.1.1.3 Ribavirin (RBV)

The RBV (1-α-D-ribofuranosyl-1,2,4-triazole-3- carboxamide) is a guanosine analog and the first drug found to inhibit MNV and human NoV replication in 2007. It was originally used as clinical treatments for HCV, Lassa fever, respiratory syncytial virus, and hepatitis E viruses (Snell, 2001). The EC50 was 40 μM for Norwalk replicon and had 82% reduction of replicon genome at 100 μM [33, 44]. However, since some studies found RBV had poor inhibition of MNV and Norwalk replicon, coupled with numerous adverse effects, it is not a desirable NoV antivirals [45].

3.1.1.4 CMX521

CMX521 is a novel antiviral drug discovered by the Chimerix. The press release reported that it is at phase I clinical trial and has potent antiviral activity against NoV. Pharmacokinetics, safety, tolerability were evaluated in approximately 50 healthy adults [33, 46].

3.1.1.5 Non-nucleoside inhibitors

3.1.1.5.1 Suramin and related derivative

Suramin is a sleeping sickness medication that was found to effectively inhibit in vitro activities of MNV and human polymerases [47]. NF03, which is the smaller

derivative of suramin, could inhibit mouse and human NoV RdRp activities with IC50s of 200 and 71.5 nM, respectively [47]. NF023 was also a smaller suramin derivative that could inhibit both human and mouse NoV RdRp activities. In addition, naphthalene disulfonate (NAF2) and tetrasodium (PPNDS) were also found to be capable of inhibiting human NoV RdRp [33, 48, 49]. However, despite of the promising effectiveness, the antiviral efficacy of these compounds are reduced due to the poor bioavailability and cell permeability [33, 48, 50, 51] Another study also identified some compounds (NIC02 [2.2.11], NIC04, NIC10, and NIC12) by using viral enzyme activity assays. MNV replication and Norwalk replicon were both found to be inhibited [33, 52].

3.1.2 Protease inhibitors

Rupintrivir is one of the protease inhibitors (PIs) discovered for NoV. In addition to the potential efficacy of this compound, it also possesses broad-spectrum antiviral effects against coronaviruses, picornaviruses, and caliciviruses such as FCV, MNV, and Norwalk replicon [33, 53]. However, it was also found that Rupintrivir had limited bioavailability and pharmacokinetics due to the low potency *in vivo*. As a result, the Rupintrivir may not be an ideal therapy for the NoV infection [33, 47].

3.1.3 Protein targets

Deubiquitinase (DUB) inhibitor is one of the recently discovered NoV antivirals that can regulate ubiquitin-ubiquitin-proteasome system [33, 54–57]. WP1130 [2.4.1] is a small synthetic DUB inhibitor found to inhibit NoV and MNV replication through unfolded protein response. However, due to the low bioavailability, show that MNV inhibition was limited to small intestine in mice [33, 56].

3.1.4 Immunomodulators

Immunomodulators are found to be a potent therapeutic antiviral for NoV since they could induce powerful host response. Interferons are one of the best immunomodulators and many studies have shown that type I, type II IFNs, and their receptors could provide protection against human and murine NoV infections [33, 58–66]. The type III IFNs (IFN-λ) has been recently explored and was shown to be capable of preventing persistent MNV infection in mice system [33, 67, 68].

3.1.5 Compounds with unknown targets

Currently, the only NoV antiviral candidate that completed clinical trials is called NTZ. The NTZ was found to have a good antimicrobial activity against various bacterial, viral, and protozoan infections. It is also an FDA-approved drug for the treatment of *Giardia* and *Crytosporidium* infections [33, 69]. Treatment of NTV was found to reduce durations of gastroenteritis symptoms when compared to the placebo (P = 0.0295) in the phase II randomized double-blind trial [33, 70]. Another study also evidenced that NTZ was capable of curing immunosuppressed transplant patient with NoV infection and 10 consecutive days of gastroenteritis symptoms [33, 71]. It was also recently found that NTZ could potently inhibit GI NoV replicon at a clinically relevant concentration (5 ug mL^{-1}) [33, 72].

NoV in stool samples from a pediatric patient with chronic NoV following kidney transplantation was also shown to be cleared [73]. There have been some discrepancy regarding the effectiveness of NTZ for NoV treatment [33, 74–77].

However, since NTZ is currently the only therapeutic method except from RBV and immunoglobulins, it is still supportive to NoV infected-patients.

3.2 Remarks on gastroenteritis symptoms in coronavirus

Acute gastroenteritis symptoms including diarrhea, vomiting and abdominal pain are not only limited to NoV infections. Viruses such as rotavirus, astrovirus, calicivirus are common etiologic agents for acute gastroenteritis [78]. Coronaviruses, which is the genus of the most concerned issue of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic in the world, is also identified as causative agent of diarrhea [78]. It was reported that approximately 12% of COVID-19 patients experienced gastrointestinal symptoms based on a systematic review and meta-analysis of published studies from November 1, 2019 to March 30, 2020 [79]. One study observed mild initial gastrointestinal symptoms (e.g., diarrhea, abdominal pain, nausea, and vomiting) preceded the fever and respiratory problems. The study also found the fecal test remained positive post 12 day of disease onset. Longer durations between symptom onset and fecal virus-positive and viral clearance were also observed [80, 81]. Another study also found gastrointestinal symptoms such as diarrhea (24.2%), anorexia (17.9%), and nausea (17.9%) occurred in COVID-19 patients [80, 82]. Results from a systematic meta-analysis study also showed the incidence rate of diarrhea from 2–50% of the COVID-19 positive cases [80, 83]. Furthermore, according to clinical cases, intestinal damages were found to be manifested after respiratory symptoms [80, 84].

Taken together, since gastroenteritis symptoms could commonly occur in various virus-infected individuals. It is not easy to differentiate if it is caused by the SARS-CoV-2 by simply observing the symptoms of patients. It was also indicated that patients with gastroenteritis symptoms also take a longer time to present to healthcare systems and to be confirmed after diagnosis [85]. Further detection of SARS-CoV-2 RNA or diagnosis of other symptoms correlated with SARS-CoV-2 disease onset are required. Precautions should also be taken carefully for the infectiveness and transmissibility of SARS-CoV-2 in COVID-19 positive feces in stool [80].

4. Conclusions

NoV has been a major cause leading to acute gastroenteritis and hundreds of thousands mortalities annually. Also, significant economic and social impacts have been resulted from this pathogen despite preclinical or clinical research are intensively ongoing. Technical issues such as the limitations in the current used human culture systems need to be overcome for the development of effective vaccines or drugs. Epidemiological studies also suggest that development of multivalent vaccines for both GI and GII NoV are the only solution for broad-spectrum and effective protection. As mentioned previously in this chapter, it is promising that several vaccines have gone through clinical trials and many drugs are currently in clinical use. However, it is of note that since clinical trials mainly enrolled adults, it would be necessary to evaluate the safety and effectiveness of the candidate vaccines in all age groups since NoV poses greater threats to children and elderly groups. To facilitate the progress of vaccine/drug developments for NoV antivirals, exploration of the relationship between viral strains and host human immunogenicity and antigen types at clinical practices would also be helpful. Improvements in diagnostic methods and outbreak containment or management may help to alleviate the epidemics.

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Conflict of interest

The authors declare no conflict of interest.

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