Pathogenesis of Human Norovirus Genogroup II Genotype 4 in Post-Weaning Gnotobiotic Pigs^S

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Norovirus is the most common cause of acute gastroenteritis. Its pathogenesis is poorly understood owing to the difficulty of establishing viral infection in animal models. Here, post-weaning gnotobiotic pigs were infected with human norovirus genogroup II genotype 4 (HuNoV GII.4) to investigate the pathogenesis and replication of the virus. Three groups of four pigs were infected with 1×10^5 , 1×10^6 , or 1×10^7 genomic equivalent (GE) copies of HuNoV GII.4. Four pigs were used as negative controls. Blood and rectal swab samples were collected after viral infection, and gross lesions were examined after necropsy. Diarrhea was induced in 25% and 75% of pigs infected with 1×10^6 and 1×10^7 GE copies, respectively. Viral shedding was detected in 50%, 75%, and 50% of pigs infected with 1×10^5 , 1×10^6 , and 1×10^7 GE copies, respectively. Viremia was detected in 25% of pigs infected with either 1×10^6 or 1×10^7 GE copies. When gross lesions of gastroenteritis were investigated, the ileum walls of the infected pigs were thinner than those of the controls. Villi atrophy and inflammatory cell infiltration were identified in the ileum of each infected pig. Viral capsid was identified in the jejunum, ileum, colon, spleen, and mesenteric lymph node. Virus replication was newly verified in the spleen and mesenteric lymph nodes by detection of negative-sense viral RNA. In conclusion, HuNoV GII.4 could induce acute gastroenteritis and replicate in the extra-intestinal lymphoid tissues in post-weaning gnotobiotic pigs. Therefore, such pigs would be a suitable animal model for studying the pathogenesis and replication of HuNoV.

Keywords: Norovirus, gnotobiotic pig, gastroenteritis, pathogenesis, replication

Introduction

Viral gastroenteritis caused by human norovirus (HuNoV) infection is a serious foodborne disease worldwide. HuNoV, which belongs to the family *Caliciviridae* and the genus *Norovirus*, causes 23 million cases and accounts for over 50,000 hospitalizations per year in the USA [1–4]. Norovirus can infect people of all ages, and infection often follows the consumption of virus-contaminated water or foods [1, 5–7]. In particular, HuNoV genogroup II genotype 4 (HuNoV GII.4) is the major causative agent of most cases of infection in Korea [8, 9]. Since a preventive

vaccine is not currently available, the only way of preventing HuNoV infection is consumption of chemically treated clean tap water and heat-treated foods [10–12]. The pathogenesis and replication of HuNoV is poorly understood because cell-culture systems and proper animal infection models are not available [13, 14].

Gnotobiotic pigs have been used as animal models for the study of pathological features and immunological responses to HuNoV infection. They are useful as infection models because they have not been exposed to pathogens and have histo-blood group antigens (HBGAs) that are similar to those of humans [15–17]. Most of the initial

studies of the pathogenesis of HuNoV have been conducted using newborn gnotobiotic piglets. Those studies provide fundamental information about norovirus infection, such as the optimal titer of the virus, the viral shedding period, the induction of viremia, and the target cells of the virus [16]. Post-weaning gnotobiotic pigs have also been used to reproduce the clinical features of HuNoV [18]. It was suggested that the maturation status of immunological and physiological systems of post-weaning gnotobiotic pigs would be different from that of newborn piglets [18]. Therefore, it is reasonably expected that the experimental infection of post-weaning gnotobiotic pigs rather than new-born piglets with HuNoV would reflect infection in young children who are one of the high risk groups for norovirus infections.

There are several controversies pertaining to the tropism of HuNoV and murine norovirus (MuNoV). Intestinal epithelial cells are known to be the major target of HuNoV. This concept is supported by recent studies that show replication of HuNoV in stem cell-derived human enteroids and in intestinal tissue from immunocompromised patients [19, 20]. Recently, macrophages, dendritic cells, B cells, and T cells in the gut-associated lymphoid tissue have been suggested as the targets of MuNoV [21–24]. Human B cells were once regarded as potential targets of HuNoV, but other studies could not reproduce these findings [23, 25]. Interestingly, it has been found that MuNoV can infect lymphoid organs such as the spleen [26, 27]. A recent study also demonstrated the presence of viral capsid protein and RNA genome in lymphatic organs such as tonsil, spleen, and mesenteric lymph nodes of pigs infected with HuNoV [28]. However, definitive evidence of HuNoV replication in lymphoid organs seems to require further studies.

In this study, we attempted to define the pathological features and plausible replication organs of HuNoV GII.4 in post-weaning gnotobiotic pigs. HuNoV GII.4 readily induces acute gastroenteritis in post-weaning gnotobiotic pigs, as expected. For the first time, we present evidence of viral replication in lymphoid organs by demonstrating negative-sense viral RNA (NS vRNA), a replicative intermediate antigenome of norovirus. We expect that our results will meaningfully contribute to improved understanding of the pathogenesis and replication of HuNoV.

Materials and Methods

Virus Preparation

HuNoV strain KU131206—HuNoV GII.4—obtained from a patient suffering from acute gastroenteritis was used as the

infectious agent. The virus stock was diluted 10-fold with minimum essential media (Wellgene, Korea). The diluted solutions were centrifuged at $3,000 \times g$ for 30 min, and the supernatant was collected. RNA was extracted from the supernatant using a QIAamp viral RNA extraction kit (Qiagen, USA), and the viral RNA was stored at -70°C .

Animals and Experimental Design

A total of 16 4-week-old, post-weaning gnotobiotic pigs were provided by the Bio-organ Research Center, Konkuk University, Seoul, Korea. The experimental procedures were approved by the Institutional Animal Care and Use Committee, Konkuk University, Korea (approval number KU14073). The pigs were adapted for three days at a biosafety level 2-grade facility by providing commercial feed before conducting experiments. All infection experiments were carried out at the biosafety level 2-grade facility located at the College of Veterinary Medicine, Konkuk University. Three groups of pigs (four pigs per group) were orally inoculated with 1×10^5 , 1×10^6 , or 1×10^7 genomic equivalent (GE) copies of HuNoV GII.4. Four pigs were used as negative controls. Blood and rectal swab samples were collected every day for 3 days after viral infection. All of the pigs were euthanized at day 3 with potassium chloride after administering zolazepam. Gross pathogenic lesions of the small and large intestines, mesenteric lymph nodes, and spleen were examined. The collected organs were stored at -70°C and fixed in 4% formaldehyde.

Clinical Signs and Preparation of Fecal and Serum Samples

Clinical features of gnotobiotic pigs infected with HuNoV were determined by the severity of diarrhea for 3 days after infection. The rectal swab samples collected from the gnotobiotic pigs were diluted 1:10 (w/v) with phosphate-buffered saline (PBS). The fecal samples were placed in 1.5-ml microcentrifuge tubes and 500 μl of PBS was added. All the diluted samples were centrifuged at $3,000 \times g$ for 30 min, and supernatants were collected. The blood samples were collected from the jugular veins of the gnotobiotic pigs, and the serum samples were separated by centrifugation at $2,000 \times g$ for 10 min. Viral RNA was extracted from the supernatants from the fecal and serum samples using a QIAamp viral RNA extraction kit (Qiagen).

Detection of Norovirus in Fecal, Serum, and Tissue Samples

Semi-nested RT-qPCR was conducted using a T100 Thermal Cycler (BioRad, USA) to detect norovirus in the fecal and serum samples. The first RT-qPCR was conducted with a total volume of 20 μl consisting of 10 pmol of each GII-forward 1 (GII-F1) (5'-GGG AGG GCG ATC GCA ATC T-3') and a GII-reverse 1 (GII-R1) (5'-CCR CCI GCA TRI CCR TTR TAC AT-3') primer, 5 μl of RNA sample, and a Maxime RT-PCR premix kit (iNtRON Biotechnology, Korea). The first RT-qPCR conditions were: 45°C for 30 min; 94°C for 5 min; 25 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 10 min. The second PCR was conducted using 2 μl of the first PCR product

and a GII-F3 (5'-TTG TGA ATG AAG ATG GCG TCG ART-3') and a GII-R1 primer in a Maxime PCR premix kit (i-star taq) (iNtRON Bio-technology), and the amplification reaction was expected to produce a 310-bp product. The second PCR conditions were: 94°C for 5 min; 25 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 10 min. For detection of NS vRNA, cDNA was synthesized as follows: 10 µl of RNA mixture was prepared with 10 pmol of GII-F1 primer, 8 µl of RNA extracted from spleen and mesenteric lymph node, and 1 µl of 10 mM dNTP mix. The RNA mixture was incubated at 65°C for 5 min and then placed on ice for 1 min. The cDNA synthesis mixture was prepared by adding 2 µl of RT buffer to the RNA mixture, with 4 µl of 25 mM MgCl₂, 2 µl of 0.1 M DTT, 1 µl of RNaseOUT, and 1 µl of SuperScript III RT (Invitrogen, USA). The cDNA synthesis mixture was incubated at 50°C for 50 min and then at 85°C for 5 min. Finally, 1 µl of RNase H was added and incubated at 37°C for 20 min. The first PCR amplification was conducted using 5 µl of the cDNA and a GII-F1 and a GII-R1 primer pair in a Maxime PCR premix kit (i-star taq) (iNtRON Bio-technology). The PCR conditions were: 94°C for 5 min; 25 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec; with a final extension at 72°C for 10 min. The second PCR conditions were the same as described above.

Determination of Viral Dose

Quantitative RT-qPCR was used to determine the GE copy of HuNoV GII.4. The reaction was conducted in a 25 µl total reaction volume containing RNase-free water, 5 µl RNA sample, 12.5 µl of 2 × OneStep RT-PCR Buffer III (Takara, Japan), 0.5 µl of Ex Taq HS (Takara), 10 pmol of each primer (QNIF2d and COG2R), and 5 pmol of probe (QNIFS) by using previously verified primer and probe sequences [29, 30]. The RT-qPCR was performed using a SmartCycler II system (Cepheid, USA) as follows: reverse transcription at 42°C for 5 min; an initial denaturation at 95°C for 10 sec; a two-step amplification of 45 cycles; denaturation at 95°C for 15 sec; and annealing and extension at 60°C for 30 sec. The GE copy of the virus was calculated based on a standard curve constructed using synthetic HuNoV GII RNA (ATCC, USA).

Hematoxylin and Eosin Staining, and Immunohistochemistry

The jejunum, ileum, colon, spleen, and mesenteric lymph nodes from each pig were placed in 4% neutral-buffered formaldehyde and embedded in paraffin by standard methods. Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) assays with the tissue samples were conducted as described previously [28].

Results

Viral RNA Detection in Serum and Fecal Samples

As expected, norovirus RNA was not detected in either fecal or serum samples from the negative control pigs (Table 1). Viremia was not detected in the pigs infected with 1×10^5 GE copies of norovirus for the 3 days post-infection (dpi). However, viremia was detected in one out of four (1/4; 25%) of the pigs infected with 1×10^6 GE copies of norovirus at 3 dpi and in 1/4 (25%) of the pigs infected with 1×10^7 GE copies at 1 dpi (Table 1). Viral shedding was detected in the fecal samples from 2/4 (50%), 3/4 (75%), and 2/4 (50%) of the pigs infected with 1×10^5 , 1×10^6 , and 1×10^7 GE copies of norovirus during the experimental period, respectively (Table 1). These results indicated that HuNoV successfully infected the post-weaning gnotobiotic pigs and was excreted in their feces. Furthermore, systemic infection was induced by viral doses of more than 1×10^6 GE copies of the norovirus in those pigs.

Clinical Manifestations and Microscopic Lesions

Clinical manifestations were evaluated for 3 days after norovirus infection according to the severity of diarrhea (Fig. S1). As expected, the negative control pigs did not experience diarrhea. Furthermore, diarrhea was not observed in the pigs infected with 1×10^5 GE copies of norovirus (Table 2). Mild diarrhea was observed in only

Table 1. Number of pigs containing norovirus RNA determined from fecal and serum samples.

Virus titer ^a	Negative		1×10^5		1×10^6		1×10^7	
	Serum	Feces	Serum	Feces	Serum	Feces	Serum	Feces
0 dpi ^b	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
1 dpi	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	3/4 (75%)	1/4 (25%)	1/4 (25%)
2 dpi	0/4 (0%)	0/4 (0%)	0/4 (0%)	2/4 (50%)	0/4 (0%)	2/4 (50%)	0/4 (0%)	1/4 (25%)
3 dpi	0/4 (0%)	0/4 (0%)	0/4 (0%)	1/4 (25%)	1/4 (25%)	2/4 (50%)	0/4 (0%)	0/4 (0%)
Total ^c	0/4 (0%)	0/4 (0%)	0/4 (0%)	2/4 (50%)	1/4 (25%)	3/4 (75%)	1/4 (25%)	2/4 (50%)

^aVirus titer: viral genomic equivalent (GE) copy number

^bdpi: days post-infection

^cTotal: the number of pigs with viremia in the serum and fecal shedding of the virus detected by reverse transcription polymerase chain reaction (RT-qPCR) in the present study

Table 2. The number of pigs with clinical signs of diarrhea.

Virus titer ^a	Negative	1 × 10 ⁵	1 × 10 ⁶	1 × 10 ⁷
0 dpi ^b	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
1 dpi	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
2 dpi	0/4 (0%)	0/4 (0%)	1/4 (25%)	2/4 (50%)
3 dpi	0/4 (0%)	0/4 (0%)	0/4 (0%)	3/4 (75%)
Total ^c	0/4 (0%)	0/4 (0%)	1/4 (25%)	3/4 (75%)

^aVirus titer: viral genomic equivalent (GE) copy number
^bdpi: days post-infection
^cTotal: the number of pigs with clinical signs of diarrhea during the present study

one pig (25%) infected with 1 × 10⁶ GE copies of norovirus at 2 dpi (Table 2). Of the pigs infected with 1 × 10⁷ GE copies of norovirus, two animals (50%) produced severe watery diarrhea at 2 dpi, and one (25%) had late-onset mild diarrhea at 3 dpi (Table 2); therefore, collectively, there was diarrhea in 75% of the pigs. These results clearly demonstrated that the typical clinical signs of acute gastroenteritis could be dose-dependently induced in post-weaning gnotobiotic pigs infected with 1 × 10⁶ and 1 × 10⁷ GE copies of HuNoV. Gross pathological lesions were identified in the ilea of the infected pigs, but not in the negative controls. The ilea walls of the infected pigs were thinner than those of the negative controls (Fig. S2). Furthermore, the mesenteric lymph nodes of infected pigs were congested (data not shown). Microscopic pathological examination revealed villi atrophy and infiltration of inflammatory cells in the ilea of the infected pigs, but not in the negative controls (Fig. 1). H&E straining did not reveal

Fig. 1. Histological examination of the ilea from gnotobiotic pigs. Pathological observations were obtained by H&E staining of the ileum samples obtained from representative pigs. (A) A negative control (pig #483) and pigs infected with (B) 1 × 10⁵ (pig #486), (C) 1 × 10⁶ (pig #493), and (D) 1 × 10⁷ (pig #495) GE copies of human norovirus. Magnification × 200. Size bar represents 100 μm.

any other abnormal features in the other organs examined in the present study.

Detection of Norovirus Capsid Protein and Negative-Sense Viral RNA

The jejunum, ileum, colon, spleen, and mesenteric lymph nodes collected from the pigs in the experimental groups were examined to detect norovirus capsid protein (Fig. 2

Fig. 2. Detection of human norovirus capsid antigen in gnotobiotic pigs. Capsid protein was determined by IHC from the intestinal and lymphoid tissue samples obtained from representative pigs. (A) Jejunum, (B) ileum, (C) colon, (D) spleen, and (E) mesenteric lymph node of a negative control pig (pig #483). (F) Jejunum (pig #472), (G) ileum (pig #474), (H) colon (pig #495), (I) spleen (pig #495), and (J) mesenteric lymph node (pig #474) of pigs infected with human norovirus. Magnification × 200.

Table 3. Detection of viral capsid protein and negative-strand viral RNA in pigs infected with norovirus.

Virus dose	Pig No.	Viral capsid protein					NS vRNA ^a	
		JE ^b	IL ^c	CO ^d	SP ^e	ML ^f	SP	ML
Control	483	-	-	-	-	-	-	-
	484	-	-	-	-	-	-	-
	491	-	-	-	-	-	-	-
	492	-	-	-	-	-	-	-
1×10^5	473	+	+	-	+	-	+	+
	474	-	++	-	+	++	+	+
	485	++	+	-	-	+++	-	-
	486	+	++	-	-	-	-	-
1×10^6	471	-	-	+	-	+	+	+
	472	++	+	+	++	+	+	+
	493	-	-	-	-	+	-	-
	494	+	++	+	-	+	-	+
1×10^7	475	-	-	+	-	-	-	-
	476	-	-	+	-	-	-	-
	495	+++	+	+	++	-	+	-
	496	-	-	++	-	++	+	+

^aNS vRNA: Negative-sense viral RNA^bJE: Jejunum, ^cIL: Ileum, ^dCO: Colon, ^eSP: Spleen, and ^fML: Mesenteric lymph node

and Table 3). The viral capsid protein was not detected in any of the tissues collected from the negative control pigs; however, it was identified in all the examined tissues from the norovirus-infected pigs, according to IHC results (Fig. 2). In the pigs infected with 1×10^5 GE copies of norovirus, viral capsid was detected in the jejunum (75%), ileum (100%), spleen (50%), and mesenteric lymph nodes (50%), but was not detected in the colon (Table 3). In pigs infected with 1×10^6 GE copies of norovirus, viral capsid was detected in the jejunum (50%), ileum (50%), colon (75%), spleen (25%), and mesenteric lymph node (100%) (Table 3). In pigs infected with 1×10^7 GE copies of norovirus, the viral capsid was detected in the jejunum (25%), ileum (25%), colon (100%), spleen (25%), and mesenteric lymph nodes (25%). The viral capsid protein was particularly prevalent in the large intestine tissues, depending on the viral dose (Table 3). Viral capsid proteins were located at both the epithelial layers and the lumina of the ilea villi. Capsid proteins were also found in lymphoid organs, such as spleen and mesenteric lymph nodes, and H&E-stained cells had lymphocyte-like and monocyte-like morphological features (Fig. 3). In order to demonstrate viral replication, the NS vRNA as a replicative

Fig. 3. Determination of cell types infected with human norovirus in gnotobiotic pigs.

Capsid protein was identified by IHC and the cell types were determined by morphological features. (A) Jejunum (pig #495), (B) colon (pig #496), (C) spleen (pig #495), and (D) mesenteric lymph nodes (pig #496) of pigs infected with 1×10^7 GE human norovirus. Arrows and arrow heads indicate macrophage-like and lymphocyte-like cells, respectively. Magnification $\times 400$. Size bar represents 50 μ m.

intermediate anti-genome was detected in the RNA samples extracted from the spleen and mesenteric lymph nodes of the pigs infected with HuNoV (Table 3). In the pigs infected with 1×10^5 GE copies of the virus, NS vRNA was determined in 50% of the spleen and mesenteric lymph node samples. NS vRNA was detected in 50% of the spleen and 75% of the mesenteric lymph nodes of pigs infected with 1×10^6 GE copies of the virus. NS vRNA was found in 50% of the spleen and mesenteric samples, and in 25% of the lymph nodes of pigs infected with 1×10^7 GE copies of the virus.

Discussion

Norovirus is one of the most important causes of acute gastrointestinal infection. However, the mechanism by which HuNoV infection occurs remains unclear because proper cell culture systems and animal infection models have been poorly established [1, 13, 14]. Several approaches to develop a cell culture system for HuNoV infection have been attempted including three-dimensional culturing [13, 19, 23]. There has also been considerable focus on using MuNoV as an alternative model for understanding the pathogenesis and immune response of HuNoV [22, 26, 27,

31]. Since gnotobiotic pigs have HBGAs similar to those of humans and have no experience of exposure to environmental antigens, they have also been used as an alternative animal model for the study of HuNoV pathogenesis [17, 32, 33]. In the present study, a HuNoV infection model was established in post-weaning gnotobiotic pigs. Acute gastroenteritis, a typical sign of norovirus infection in humans, was dose-dependently induced in the post-weaning gnotobiotic pigs infected with HuNoV during a short experimental period. Similarly, post-weaning gnotobiotic pigs were challenged with HuNoV to verify the viral infectivity and fecal shedding periods [18]. The study demonstrated the induction of diarrhea and fecal viral shedding within 2 days post infection as clinical features of HuNoV infection in pigs. Therefore, these results collectively verify that HuNoV could infect and reproduce acute gastroenteritis in post-weaning gnotobiotic pigs.

In the present study, the infectivity of HuNoV was examined using three different doses: 1×10^5 GE, 1×10^6 GE, and 1×10^7 GE. There was also an attempt to confirm whether HuNoV causes viremia in post-weaning pigs. Viremia could not be demonstrated in pigs infected with 1×10^5 GE. However, viremia was observed in pigs infected with 1×10^6 GE at 3 dpi and in those infected with 1×10^7 GE at 1 dpi. Fecal viral shedding was demonstrated in all three groups, although pigs infected with 1×10^6 GE and 1×10^7 GE exhibited earlier shedding than pigs infected with 1×10^5 GE. These results indicated the dose-dependent infectivity of HuNoV in post-weaning gnotobiotic pigs. Similar results were reported in another study, in which infection of HuNoV was only proved in post-weaning gnotobiotic pigs infected with a higher dose of the virus, but not in pigs infected with a lower dose [18]. Therefore, these results indicate that a higher dose of HuNoV is more effective for inducing infection and eliciting clinical signs in gnotobiotic pigs than a lower dose. No clinical signs were detected in the pigs infected with 1×10^5 GE. However, diarrhea was induced in pigs infected with 1×10^6 GE and 1×10^7 GE from 2 dpi. The most severe diarrhea was observed in pigs infected with 1×10^7 GE. Therefore, it seems that the clinical signs of norovirus infection were also dose-dependent.

When the internal organs of the pigs were examined, the walls of the ilea of the infected pigs were thin, whereas those of the negative control pigs were normal, without any pathological gross lesions (data not shown). Therefore, microscopic lesions of the ilea were investigated to determine the severity of the pathological changes in the

infected pigs. The villi of the small intestines in the infected pigs were significantly shorter than those of the negative controls. As expected, the most severe villi atrophy occurred in the pigs infected with the higher dose (1×10^7 GE), and less severe lesions were detected in the pigs infected with the lower doses of the virus (1×10^6 GE and 1×10^5 GE). Therefore, it was assumed that the degree of villi atrophy contributed to the severity of the diarrhea. The IHC assay revealed HuNoV capsid proteins in the jejunum, ileum, colon, spleen, and mesenteric lymph nodes of pigs infected with the HuNoV. The detection rates of the viral capsid antigen were particularly high in the large intestines of infected pigs, and detection was dose-dependent. Viral antigens were located at both the epithelial region and inside the villi. These results implied that the virus may penetrate the basal laminar layer beneath the epithelium.

Several studies have revealed that MuNoV and HuNoV can be carried through the basal lamina by M cells, and cause tropism to macrophages, dendritic cells, and B cells [22, 24, 27, 34]. The morphological features of cells stained with anti-HuNoV capsid antibody in the jejunum, ileum, colon, spleen, and mesenteric lymph nodes were determined to identify the types of infected cells. The cells stained during the IHC assay appeared to have the morphologic features of lymphocytes and monocytes. Similar results were obtained from the detection of viral antigens in dendritic cells and B lymphocytes of norovirus-infected chimpanzees [35]. The presence of the lymphocyte-like and monocyte-like morphological features suggested that some immune cells might be infected with HuNoV, as with MuNoV, which can infect various immune cells [22, 24, 27, 36]. Despite the evidence for HuNoV infection of immune organs, there are still controversies as to whether B lymphocytes might be the potential targets of HuNoV, or not [20, 23, 25, 37]. As RAG/IL2RG-deficient gnotobiotic pigs infected with HuNoV also showed that depletion of immune cells could induce prolonged HuNoV infection, the role of immune cells in HuNoV infection and pathogenesis remains complicated [37]. However, the detection of NS vRNA in this study indicated that HuNoV could infect and replicate in lymphoid organs such as the spleen and lymph nodes. The limitation of this study was not providing definitive evidence of the target cells of HuNoV. We suggested the monocyte-like and lymphocyte-like cells in such lymphoid organs might be target cell candidates because they had the capsid protein of HuNoV.

In conclusion, infections with HuNoV GII.4 induced acute gastroenteritis in post-weaning gnotobiotic pigs,

along with fecal shedding and viremia. The virus could infect both the intestinal tract and lymphatic organs, such as spleen and mesenteric lymph nodes, in which viral replication was verified. Further studies are required to determine how HuNoV infects lymphoid tissues, and its effects on viral pathogenesis.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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