한국가축위생학회지 제43권 제4호 (2020)
Korean J Vet Serv, 2020, 43(4), 237-244
ISSN 1225-6552, eISSN 2287-7630
https://doi.org/10.7853/kjvs.2020.43.4.237

Korean Journal of
Veterinary Service
Available online at http://kjves.org

< Original Article >

Development of sandwich enzyme-linked immunosorbent assay for a large-scale detection of porcine transmissible gastroenteritis virus in feces

Yeonsu Oh[1], Sang-Joon Lee[1], Ho-Seong Cho[2], Dongseob Tark[3]*

[1]Department of Veterinary Pathology, Collage of Veterinary Medicine and Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Korea
[2]College of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan 54596, Korea
[3]Korea Zoonosis Research Institute, Jeonbuk National University, Iksan 54531, Korea

(Received 24 November 2020; revised 15 December 2020; accepted 17 December 2020)

Abstract

Porcine transmissible gastroenteritis (TGE) has been a significant cause of economic losses in pig farming industry since 1950s. Although transmissible gastroenteritis virus (TGEV) has declined in recent years, it should not be excluded because of its characteristics; the frequency of gene mutation, the mortality in piglets, and the possibility for sudden incidence. Therefore, the herd-level monitoring of the virus is important to prevent further circulation of TGE. The aim of this study is to develop a large-scale sandwich enzyme-linked immunosorbent assay (ELISA) with high specificity to rapidly detect TGEV in feces by using monoclonal antibodies (Mabs). The TGEV specific Mabs were produced in hybridoma cells. Among the Mabs belonged to the IgG class developed by this study, the final selected 8H6, 1B7, 4G3, and 1F8 were identified to have the neutralization ability against TGEV. The sandwich ELISA was established using 8H6 as a reporter antibody and 1B7 and the reported 5C8 as a capture antibody. The developed sandwich ELISA was able to distinguish TGEV from other pathogenic diarrheal agents (porcine rotavirus, porcine reovirus, porcine epidemic diarrhea virus (PEDV), *E. coli*, and *C. perfringens*) in tissue culture as well as fecal samples. And the detection rate of TGEV in feces was 80% compared with RT-PCR. The results suggested that the developed sandwich ELISA may be useful in the herd-level monitoring for effective preventive measures due to the early diagnosis of TGEV using a large amount of samples.

Key words : Porcine transmissible gastroenteritis virus, Sandwich ELISA, Herd-level monitoring

INTRODUCTION

Diarrhea in swine is acting as one of the biggest factors which reduce productivity in the industry. Several types of gastrointestinal diseases with diarrhea occur throughout the year and cause significant economic losses to pig farms. Pathogens such as bacteria, viruses, and protozoa can cause the various types of diarrhea, but the most problematic are viral diarrhea; transmissible gastroenteritis (TGE) and porcine epidemic diarrhea (PED).

In recent years, the porcine epidemic diarrhea virus (PEDV) has been recognized as one of the most notorious viruses for porcine diarrhea in terms of the number of positive cases and the economic losses. And the transmissible gastroenteritis virus (TGEV) has declined with its innocent relative, non-enteropathogenic porcine respiratory coronavirus (PRCV). However, there are reasons to monitor transmissible gastroenteritis virus periodically. First, the TGEV belongs to the genus *Alphacoronavirus*, family *Coronaviridae* like PEDV. Therefore, despite the presence of a vaccine, TGEV also continues to occur due to frequent gene mutations. Second, the mortality rate of TGEV can approach up to 100% in piglets under

*Corresponding author: Dongseob Tark, E-mail. tarkds@jbnu.ac.kr
ORCID https://orcid.org/0000-0001-7499-4253
These first two authors contributed equally to this work.

1 week of age and either infected or recovered pigs may be acting as a carrier with excreting viruses through feces for a long period of time. Finally, according to KAHIS (Korea Animal Health Integrated System), the number of TGE positive cases is 1,729 since 2012 and 1,682 for only 2014. This indicate that an explosive increase may occur without any notice and suggest that periodic monitoring may be necessary. Therefore, the herd-level monitoring of the virus is important to prevent further circulation of the disease.

The aim of this study is to develop a large-scale diagnostic method for detection of swine TGEV in feces. In order to establish an effective preventive measure, it is necessary to develop a diagnostic method capable of discriminating the causative agents of swine diarrhea such as PEDV, rotavirus infection and coliform diarrhea, and detecting TGEV quickly and accurately in a large quantity. The diagnostic methods to detect TGEV were developed in various ways (Bohac et al, 1975; Saif et al, 1977; Chu et al, 1982; Asagi et al, 1986; Van Nieuwstadt et al, 1988; Oh and Tark, 2019) including fluorescence antibody test (Pensaert et al, 1970; Black, 1971). Dulac et al. (1977) attempted to isolate TGEV from field specimens using cell culture and piglets. However, isolation and identification of TGEV by cell culture may take a long time and may not detect the causative agent. Although electron microscopy can detect the causative agents in the feces (Saif et al, 1977; Van Nieuwstadt et al, 1988), both methods have the disadvantage of requiring expertise and facilities in the inspection process. In general, the fluorescence antibody test has the advantage of being able to rapidly test the small intestine by frozen section or mucous membrane smearing. However, there are many subjective factors in the test result, and freshness materials are needed. The aim of this study was to develop a sandwich ELISA with high specificity to rapidly detect TGEV in feces by using monoclonal antibodies that specifically reacts with TGEV.

MATERIALS AND METHODS

Viruses and bacteria used in the study

Total six TGEV strains, four Korean isolates strains (NVRI 48 strain, NVRI 41 strain, WP strain, and Pyeongtaek strain) and two standard viruses (Purdue strain and Miller strain), were used in the study. The proliferation and potency of each virus was measured respectively in the swine testicular (ST) cell line (ATCC, MD, USA). Porcine endemic diarrhea virus (Wey strain and Japanese vaccine strain), porcine rotavirus (Korean isolate, OSU type), porcine reovirus (Korean field isolate), and porcine pathogenic bacteria, *Escherichia coli* (K88ac) and *Clostridium perfringens*, were used. All of the viruses and bacteria were distributed from the Animal and Plant Quarantine Agency (APQA), and Ministry for Agriculture, Food and Rural Affairs (MAFRA) in Republic of Korea.

Fecal sample collection after TGEV challenge

3-day-old SPF pigs were orally inoculated with $10^{7.0}$ $TCID_{50}$/mL of TGEV (NVRI 48 strain-10 passages), and fecal samples were collected daily. At the peak of viral infection pigs were necropsied and intestinal contents were collected to be stored at −80℃ until use. Pigs were fed with milk replacer *ad libitum* throughout experimental periods in accordance with the institutional animal ethical standards (IACUC no. JBNU 2020-0127).

Preparation of the monoclonal antibody to TGEV

Monoclonal antibodies against TGEV were produced according to the literature (Coyle et al, 1992; Oh and Tark, 2019). Briefly, when the Sf9 cell infected with pF9AH-bac was showed cytopathic effect completely, the cell was harvested, sonicated, and mixed with incomplete Freund's adjuvant (Sigma-Aldrich, MO, USA) for immunization. The prepared antigen was inoculated on the footpad of BALB/c mice under mild anesthesia. The popliteal lymph nodes were collected 10 days after inoculation, made into single cells, and fused with murine myeloma cell line P3X63 (ATCC, MD, USA). Production of TGEV specific monoclonal antibody from the

hybridoma cells was confirmed as follows: the ST cell infected with TGEV and intact ST cell were prepared and fixed with acetone. The fixed cells were first reacted with the hybridoma cell culture supernatant, followed by the rabbit anti-mouse immunoglobulins (IgG, IgA, and IgM) FITC conjugate (Sigma-Aldrich, MO, USA). The strong positive hybridoma cells for fluorescent antibodies were cultured in feeder cell plates derived from ICR mouse peritoneal cavity. Screening and cloning were performed twice in a single well containing hybridoma cells. Fully cloned hybridoma cells were stored in a liquid nitrogen tank until use.

The virus neutralization test for the selected monoclonal antibody was performed by serial two-fold dilution of antibodies with 200 $TCID_{50}$/50 μL of TGEV (Pyeongtaek strain) in a 96-well microplate. After sensitization, ST cells suspended in α-MEM medium supplemented with 5% FCS were injected into all wells and cultured in a CO_2 incubator for 4～5 days. The result was expressed as the reciprocal of the highest serum dilution factor neutralizing the 100 $TCID_{50}$/100 μL TGEV. Antibody isotyping was performed using a mouse-hybridoma subtyping kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's procedure.

Purification of the monoclonal antibody and conjugation with horseradish peroxidase

The monoclonal antibody was purified using fast protein liquid chromatography (FPLC) (Pharmacia, NJ, USA). The protein was quantified by the Micro BCA protein assay reagent kit (Pierce, MA, USA). The horseradish peroxidase (HRP) was conjugated according to the periodate coupling method (Nakane and Kawaoi, 1974). In brief, after dissolving 5 mg of HRP (peroxidase, Type VI-A) (Sigma-Aldrich, MO, USA) in 1.2 mL of distilled water, 0.3 mL of sodium periodate (0.1 M)/sodium phosphate (10 mM; pH 7.0) was added, and allowed to stand at room temperature for 20 minutes, followed by dialysis overnight in sodium acetate (1 mM; pH 4.0). The dialyzed HRP was mixed with 6.6 mg of the purified monoclonal antibody suspended in 0.5 mL of carbonate (20 mM; pH 9.5), and incubated at room temperature for 2 hours. Then, 100 μL of sodium borohydride (4 mg/mL) was added and incubated at 4°C for 2 hours, followed by dialysis in PBS. The dialyzed conjugate was dispensed in 0.1 mL aliquots and stored at −20°C until use.

Sandwich ELISA

Sandwich ELISA to detect TGEV antigen in feces or cell culture medium was developed with modification as described previously (Bernard et al, 1986; Van Nieuwstadt et al, 1988). 5C8 and 1B7 which specifically binds to TGEV spike protein, as virus capture antibodies were diluted to 4.2 μg/mL and 6.6 μg/mL, respectively in 100 μL of carbonate-bicarbonate buffer (0.05 M; pH 9.6) per well of an ELISA plate (Maxi-sorp, Nunc, Denmark) and incubated at 37°C overnight. Then, 150 μL of blocking solution (Tris, 0.01 M, pH 7.5; NaCl, 0.15 M; gelatin, 1%; horse serum, 10%) was added per well and incubated at 37°C for 1 hour. The fecal samples were diluted to 1/10 with diluting solution (Tris, 0.01 M, pH 7.5; NaCl, 0.15 M; gelatin, 1%; horse serum, 10%; Tween 20, 0.05%) and incubated at 37°C for 2 hours in the sensitized solid phase. Then, HRP-conjugated TGEV-specific monoclonal antibody was distributed. After 1 hour of incubation at 37°C, the 3,3', 5,5'-tetramethyl-benzidine (KPL, MD, USA) was added and reacted for 30 minutes. Finally, the reaction was stopped and the absorbance was measured at 450 nm using a microplate reader (Tecan, Mannedorf, Switzerland). Between all steps, washings were carried out four times with washes (0.01 M Tris pH 7.5, 0.5 M NaCl, 0.05% Tween 20), except for the blocking step, all reaction solutions were 100 μL per well. The results were obtained by adding samples to the wells (S) containing the monoclonal antibody, and the wells (B) containing no antibody. The results were divided by the absorbance of S and that of B. When the value was 2 or more, positive, inconclusive when the value was between 1.5 and 2, and negative when the value was less than 1.5. The setting of this range is based on the detection of TGEV in tissue culture with a titer greater than or equal to $10^{5.0}$ $TCID_{50}$/mL.

Reverse transcription polymerase chain reaction (RT-PCR) to detect the TGEV antigen

The RT-PCR was performed as a control for the TGEV detection effect by the developed sandwich ELISA. Viral RNA in feces was extracted using TRIzol® Reagent (GibcoBRL, NY, USA). For the synthesis of cDNA, the extracted viral RNA was mixed with a reverse primer (5'-TTCTAATGTAGTCGCACGCAT-3'), boiled for 5 minutes, immediately added to ice, cooled for 5 minutes and centrifuged at 10,000 rpm for 1 minute. The first strand cDNA was amplified using denatured viral RNA, 40 unit RNAsin (Promega, WI, USA), 50 mM Tris-HCl pH8.3, 3 mM $MgCl_2$, 75 mM KCl, 10 mM DTT, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dTTP, 0.4 mM dGTP and reverse primer were added to 50 μL of the reaction mixture and reacted at 50℃ for 2 minutes. Then, 4 units of reverse transcriptase (Superscript II RNase H-Reverse Transcriptase) (GibcoBRL, NY, USA) was added and reacted at 42℃ for 50 minutes. The cDNA was denatured at 95℃ for 5 minutes on a Gene Amp RT-PCR system 9600 (Perkin Elmer, MA, USA) using a reverse primer (5'-AGAACTATAGGTAACCATTGG-3') and a Thermalase Tbr Kit (Amresco, PA, USA) and then RT-PCR was performed by reacting 30 cycles of 52℃ for 45 seconds, 72℃ for 1 minute, and 95℃ for 45 seconds, followed by reaction at 52℃ for 45 seconds and 72℃ for 5 minutes. After the RT-PCR reaction was completed, the amplified DNA fragments were confirmed by electrophoresis in 1% agarose gel (containing 0.5 μg/mL ethylenebromide) at a ratio of 1/5 to 1/10 of the total reaction amount.

RESULTS

Preparation of the monoclonal antibody (Mab) to TGEV and its characterization

Four hybridoma cells were selected to produce Mab against TGEV after one step footpad immunization using recombinant TGEV spike protein. Four Mabs (8H6, 1B7, 4G3, and 1F8) were examined by fluorescent antibody test on TGEV-infected ST cells, and specific fluorescence were observed in cytoplasm (Fig. 1). The Mabs were confirmed not to react with other comparative pathogens; porcine epidemic diarrhea virus, porcine rotavirus and porcine reovirus (Table 1). As a result of isotyping the antibodies, all four Mabs were the isotype IgG class (data-not-shown). In the virus neutralization

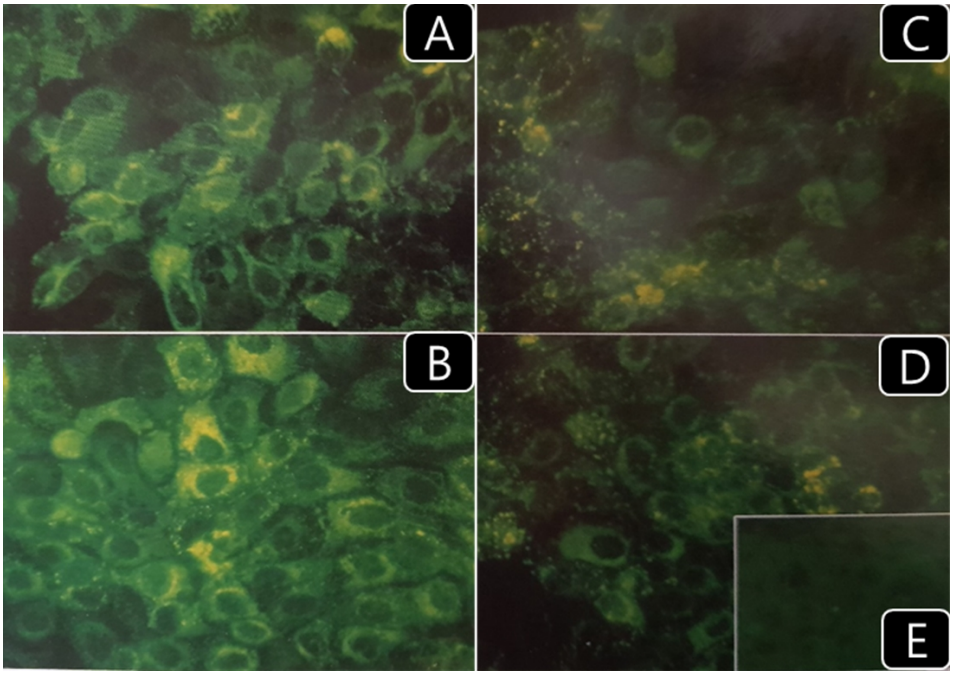

Fig. 1. Immunofluorescence patterns of the TGEV infected swine testicle cells that reacted with Mabs (A-8H6, B-1B7, C-1F8, D-4G3, E-tissue culture fluid) produced by recombinant transmissible gastroenteritis virus spike protein.

Table 1. Characterization of monoclonal antibodies (Mab) produced by using the recombinant transmissible gastroenteritis virus (TGEV) spike protein

Mab designation	Mab isotype	SN titer*	IIF†	Virus strain				
				TGE	Rota	PED	Reo	pF9AH-bac
8H6	IgG2b	640	>204800	+	−	−	−	+
1B7	IgG1	<10	25600	+	−	−	−	+
4G3	IgG2a	<10	25600	+	−	−	−	+
1F8	IgG1	<10	51200	+	−	−	−	+

*The neutralizing antibody titer was expressed as the reciprocal of the serum dilution neutralizing 100 $TCID_{50}$/mL of TGEV (Pyeongtaek strain).
†Indirect immunofluorescence test on swine testicle cell infected by TGEV (Pyeongtaek strain). The titer was determined as the last dilution that gave positive fluorescence with anti-mouse immunoglobulins conjugate FITC. Rota, Rotavirus; PED, Porcine epidemic diarrhea virus; Reo, Reovirus.

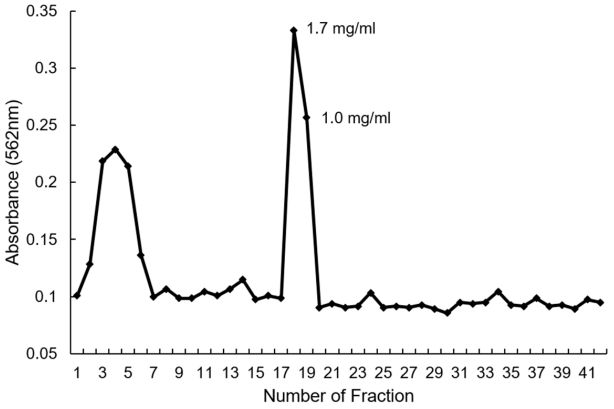

Fig. 2. Purification of monoclonal antibody (8H6) using fast protein liquid chromatography. The protein quantity of each fraction was measured by Micro BCA protein assay.

test, the 8H6 was found to produce neutralizing antibody (Table 1).

Purification of the Mab for conjugation

The Mab, 8H6 was purified using FPLC to conjugate with HRP. As shown in Fig. 2, after passing through the column, the amount of protein was peaked at fractions, 18 and 19 corresponding to be 1.7 mg/mL and 1.0 mg/mL, respectively. The two fractions were mixed and conjugated with HRP and used for the conjugated antibody.

Selection of the Mab for antigen capture

The reactivity of the newly produced three (1B7, 1F8, 4G3) and the reported one (5C8) (Chang et al, 1995) Mabs to TGEV was investigated by ELISA. As a result, 1B7 was the most suitable for capturing antigen as shown in Table 2, followed by 5C8, 1F8 and 4G3. On the other hand, when ELISA was performed by mixing various monoclonal antibodies, only the combination of two monoclonal antibodies of 5C8 and 1B7 showed high reactivity, but the other mixed solutions showed lower reactivity than that of Mab alone. Therefore, in the subsequent ELISA, a mixture of 5C8 and 1B7 was selected as a monoclonal antibody to capture the antigen.

Table 2. Efficiency of sandwich ELISA for detection of transmissible gastroenteritis virus

Capture antibodies	Efficiency of Mab*	
	TGEV(NVRI 48)	Blank†
5C8	2.365	0.119
1B7	2.599	0.086
1F8	1.906	0.084
4G3	0.639	0.118
5C8+1B7	2.774	0.123
5C8+1B7+1F8	2.471	0.113
5C8+1B7+1F8+4G3	2.326	0.122

*Efficiency of Mab in sandwich ELISA was expressed as the absorbance (450 nm) of ELISA reaction done with Mab or cell culture fluid (Blank†) coated on the plate.

TGEV detection efficiency of the sandwich ELISA

The developed ELISA showed no cross-reactivity with diarrheal agents other than TGEV, i. e., PEDV, porcine rotavirus, *Escherichia coli* and *Clostridium perfringens*, as shown in Fig. 3. In addition, the newly developed test method proved to be able to detect TGEV in vari-

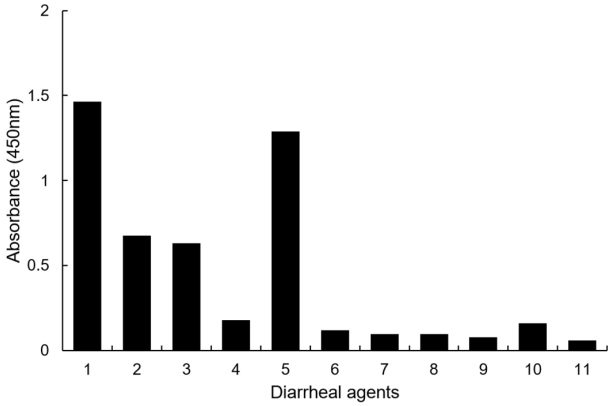

Fig. 3. Reactivity of produced Mab toward cell-cultured transmissible gastroenteritis virus and other enteric pathogens in sandwich ELISA. 1. NVRI 48 ($10^{7.0}$TCID$_{50}$/mL), 2. NVRI 41 ($10^{6.25}$TCID$_{50}$/mL), 3. WP ($10^{6.0}$TCID$_{50}$/mL), 4. Miller ($10^{3.25}$TCID$_{50}$/mL), 5. Purdue ($10^{6.5}$TCID$_{50}$/mL), 6. PED Japanese vaccine strain ($10^{5.0}$TCID$_{50}$/mL), 7. PED Wey strain ($10^{5.0}$TCID$_{50}$/mL), 8. Rota, OSU ($10^{6.0}$TCID$_{50}$/mL), 9. *E. coli*, 10. *C. perfringens*, 11. Culture medium.

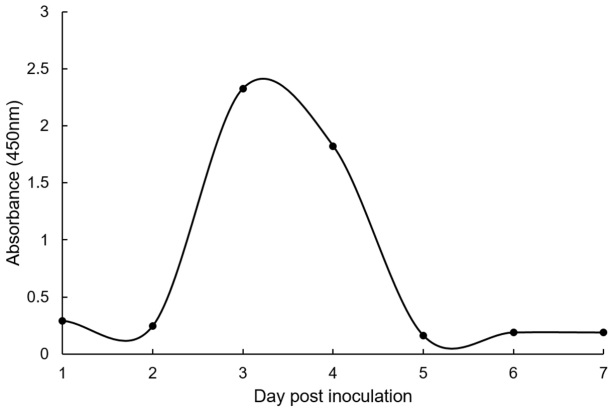

Fig. 4. Detection of TGEV by sandwich ELISA in fecal specimens from pig infected with TGEV experimentally.

ous titer, and consequently, the specificity of the diagnostic method was recognized. On the other hand, when TGEV was orally inoculated into 3-day-old pigs and the fecal samples were examined at each time point, and no virus was detected on the 1st and 2nd day of inoculation. However, the ELISA reaction reached the highest level at 3rd day and rapidly decreased at 5th days (Fig. 4). In addition, the agreement between the RT-PCR assay and the developed sandwich ELISA was about 80% (Table 3). Currently, there is almost no occurrence of TGE in domestic pig farms, virtually being impossible to get field positive samples. Therefore, 150 spike samples were prepared and tested, each of 30 spike samples for

Table 3. Comparison of sandwich ELISA and polymerase chain reaction (PCR) for detection of TGEV in fecal specimens

Sandwich ELISA	PCR		
	Positive	Negative	Total
Positive	5	3	8
Suspected	1	0	1
Negative	1	15	16
Total	7	18	25

each concentration of fecal samples from $10^7 \sim 10^3$. As a result, all positive came out except 10^3 spiked fecal samples. For the TGE negative sample, 150 fecal samples from the TGE negative farms were obtained and all of them were negative (data-not-shown).

DISCUSSION

Although porcine TGEV has been declined in recent years, it still occurs intermittently worldwide, and once it occurs, it has a short incubation period and high infectivity to the entire swine population within two to three days (Maes and Haelterman, 1979; Hsu et al, 2018; Zhang et al, 2018). Therefore, rapid, accurate, and effective herd-level monitoring is required to establish effective preventive measures. In this study, a sandwich ELISA to detect TGEV in fecal samples with various virus titer was developed, which can rapidly detect TGEV in a large number of fecal samples or cell culture fluid. Bernard et al. (1986) used three monoclonal antibodies reactive with nucleoprotein, membrane glycoprotein and spike glycoprotein, the three structural proteins of TGEV, as antigen-capture antibodies (primary antibodies) and secondary antibodies using highly immunized porcine serum were labeled with HRP to detect solid-phase immune-reactants. In the case of indirect double-antibody sandwich ELISA developed by another researcher (Van Nieuwstadt et al, 1988), rabbit serum for anti-TGEV was used for the primary antibody, three kinds of monoclonal antibodies were used for the secondary antibody, and the conjugate of anti-mouse immunoglobulin labelled with HRP was used to determine the results. In this study, the establishment of TGEV detection using monoclonal antibody alone was aimed at

minimizing the non-specific reaction by anti-TGEV polyclonal sera and enhancing the specificity of the diagnosis. In an ELISA assay for detection of virus, it is inappropriate to attach virus to an ELISA plate due to the characteristics of coronavirus when a monoclonal antibody reactive with membrane or nucleoprotein is used as an antigen capture antibody (Bernard et al, 1986; Lanza et al, 1993). Therefore, among the four newly produced monoclonal antibodies and 5C8 antibodies, the monoclonal antibody with the highest reactivity to TGEV was used as an antibody for TGEV capture (Table 2). Bernard et al. (1986) reported that sensitivity of a conjugate labeled with an enzyme to a monoclonal antibody was lower than that of a polyclonal conjugate. However, in this study, labeling HRP with purely purified monoclonal antibody using FPLC as a conjugate resulted in a significant increase in sensitivity (Figs. 2, 3). Three days old piglets were orally administered the 10th passaged TGEV (NVRI 48 strain) in ST cell line and fecal samples were collected at each time point. As a result, virus detection was maximal 3 to 4 days and not detected from 5 days after inoculation. And also the feces collected on the 3rd day showed a positive reaction in RT-PCR. These results are consistent that Van Nieuwstadt et al. (1988) also reported that the oral administration of attenuated TGEV to colostrum-deprived pigs resulted in the detection of virus from the third day after inoculation and lasted for 2 to 4 days. In addition, the feces on the third or fourth day after the virus was detected in the study was not diarrhea unexpectedly. Bernard et al. (1986) also reported that there are different in their occurrence of virus detection and diarrhea, rather virus excretion peaked before diarrhea occurs. Feces collected from experimental TGEV-inoculated pigs or TGEV-occurred farms were compared with the RT-PCR showed inconsistent results of about 16%. If RT-PCR-positive feces not detected in the ELISA were taken early (1～2 days after infection) when the virus release was at its peak, the results between the two tests would be consistent as in the experiment (Bernard et al, 1986; Van Nieuwstadt et al, 1988). In addition, the RT-PCR is possible to detect viruses with low titers because it is very sensitive detection method, however newly developed sandwich ELISA able to detect more than $10^{4.0}$～$10^{5.0}$ $TCID_{50}$/mL of virus (Fig. 3). This difference in sensitivity seems to indicate discrepancies between the two diagnostic methods. RT-PCR-negative fecal specimens that are positive for ELISA may be caused by immunoglobulin-binding factors such as rheumatoid arthritis factor or immunoglobulin-binding bacteria that may be present in the feces (Yolken and Stopa, 1979; Yolken, 1982; Van Nieuwstadt et al, 1988). In conclusion, there remains a problem of comparing the time of sample collection with the various field feces and the comparison experiment with other test methods other than the RT-PCR, the developed sandwich ELISA can be used to detect TGEV in feces or tissue culture as well as to distinguish it from other diarrheal agents. The developed sandwich ELISA will be useful for effective preventive measures due to the early diagnosis of TGEV using a large amount of samples.

The developed antigen ELISA can be used as a screening diagnostic method for large samples. However, it is recommended to perform additional confirmation by RT-PCR if infection is suspected clinically, although the developed ELISA yields a negative result due to its relatively low sensitivity. In addition, it remains a regret that comparative analysis was not possible because there is no commercial ELISA to compare and verify the developed ELISA.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ORCID

Yeonsu Oh, https://orcid.org/0000-0001-5743-5396
Sang-Joon Lee, https://orcid.org/0000-0002-2498-8397
Ho-Seong Cho, https://orcid.org/0000-0001-7443-167X
Dongseob Tark, https://orcid.org/0000-0001-7499-4253

REFERENCES

Asagi M, Ogawa T, Minetoma T, Sato K, Inaba Y. 1986. Detection of transmissible gastroenteritis virus in feces from pigs by reversed passive hemagglutination. Am J Vet Res 47(10): 2161-2164.

Bernard S, Laude H, Lantier I. 1986. Detection du coronavirus de la GET et des anticorps specifiques par une technique ELISA mixte mettant en ouvre anticorps monoclonaux et policlonaux. J Rech Porcine Fr 18: 363-370.

Black, JW. 1971. Diagnosis of TGE by FA: Evaluation of accuracy on field specimens. Proc US Anim Health Assoc 75: 492-498.

Bohac J, Derbyshire JB, Thorsen J. 1975. The detection of transmissible gastrenteritis viral antigens by immunodiffusion. Can J Comp Med 39(1): 67-75.

Chang YE, Cho SH, Kang SY. 1995. Monoclonal Antibody Production, Characterization and Utilization of porcine transmissible gastroenteritis virus. Korean Journal of Veterinary Medicine, 35 (4): 135.

Chu RM, Li NJ, Glock RD, Ross RF. 1982. Applications of peroxidase-antiperoxidase staining technique for detection of transmissible gastroenteritis virus in pigs. Am J Vet Res 43(1): 77-81.

Colye PV, Wyatt D, McCaughey C, O'Neil HJ. 1992. A simple standardised protocol for the production of monoclonal antibodies against viral and bacterial antigens. J Immunol Methods 153: 81-84.

Dulac GC, Ruckerbauer GM, Boulanger P. 1977. Transmissible gastroenteritis: Demonstration of the virus from field specimens by means of cell culture and pig inoculation. Can J Comp Med 41: 357-363.

Hsu TH, Liu HP, Chin CY, Wang C, Zhu WZ, Wu BL, Chang YC. 2018. Detection, sequence analysis, and antibody prevalence of porcine deltacoronavirus in Taiwan. Archives of virology 163(11): 3113-3117.

Lanza I, Rubio P, Muñoz M, Cármenes P. 1993. Comparison of a monoclonal antibody capture ELISA (MACELISA) to indirect ELISA and virus neutralization test for the serodiagnosis of transmissible gastroenteritis virus. J Vet Diagn Invest 5(1): 21-25.

Maes RK, Haelterman EO. 1979. A seroepizootiological study of five viruses in a swine-evaluation station. Am J Vet Res 40: 1642-1645.

Nakane PK, Kawaoi. 1974. Peroxidase-labeled antibody: Anew method of conjugation. J Histochem Cytochem 22: 1804-1091.

Oh Y, Tark D. 2019. Development of monoclonal antibody capture ELISA for the detection of antibodies against transmissible gastroenteritis virus. Korean J Vet Serv 42(1): 9-15.

Pensaert MB, Haelterman EO, Burnstein T. 1970. Transmissible gastroenteritis of swine: Virus-intestinal cell interactions. I. Immunofluorescence, histopathology and virus production in the small intestine through the course of infection. Arch Gesamte Virusforsch 31: 321-334.

Saif LJ, Bohl EH, Kohler EM, Hughes JH. 1977. Immune electron microscopy of transmissible gastroenteritis virus and rotavirus (reovirus-like agent) of swine. Am J Vet Res 38(1): 13-20.

Van Nieuwstadt AP, Cornelissen JB, Zetstra T. 1988. Comparison of two methods for detection of transmissible gastroenteritis virus in feces of pigs with experimentally induced infection. Am J Vet Res 49: 1836-1843.

Yolken RH, Stopa PJ. 1979. Analysis of nonspecific reactions in enzyme-linked immunosorbent assay testing of human rotavirus. J Clin Microbiol 10: 703-707.

Yolken RH. 1982. Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. Res Infect Dis 4: 35-68.

Zhang F, Luo S, Gu J, Li Z, Li K, Yuan W, Ye Y, Li H, Ding Z, Song D, Tang Y. 2019. Prevalence and phylogenetic analysis of porcine diarrhea associated viruses in southern China from 2012 to 2018. BMC veterinary research 15(1): 1-9.