大韓獸醫學會誌(2006) 第46卷 第4號
Korean J Vet Res(2006) 46(4) : 363~370

Serological and genetic characterization of the European strain of the porcine reproductive and respiratory syndrome virus isolated in Korea

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(Accepted: November 23, 2006)

Abstract : Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of swine that occurs all over the swine industry worldwide. It was first observed in the Unite States in 1987 then in Europe in 1990. It has been described in Japan and in Korea in 1993. PRRS virus is divided into two distinct types, North American and European, genetically. Based on our limited knowledge there has been no report on the existence of European PRRSV. But according to the government's Korea Customs Service there has been many importations of breeding pigs from Europe. These seem to make an estimate that European PRRSV could be introduced in Korea by inflow of European breeding pigs. We first detected the European PRRSV could be introduced in Korean pig farms by using polymerase chain reaction (PCR). Further, it is also identified that there are not only North American PRRSV antibody but also a European PRRSV antibody. According to the genetical and serological experiment results, the presence of established North American PRRSV in Korea is due to the use of live vaccines made of North American PRRSV strain as well field virus infection, and the European PRRSV is possibly introduced from imported breeding stock.

Key words : European PRRSV, homology, PCR (Polymerase Chain Reaction)

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of swine that occurs all over the world. It was first observed in the Unite States in 1987 then in Europe in 1990 [6]. It has been described in Japan and in Korea in 1993 [10]. In the 1980s an unrecognized disease syndrome caused heavy production losses in pig herds in North America.

Surprisingly, this disease syndrome was relatively unknown until reports have been made several outbreaks of the disease in Indiana swine herds [27, 28]. For the next 4-5 years the disease was to become popularized by “Mystery Disease” or “Mystery Swine Disease” [11]. The predominant reproductive and respiratory clinical signs associated with this syndrome have resulted in descriptive term “Swine Infertility and Respiratory Syndrome” (SIRS), which has become the most commonly used name for mystery swine disease in the United States [29]. The disease described in Europe is clinically similar to SIRS in the United States, except disease has spread much more rapidly through out the European swine industry, and cutaneous cyanosis of the ears, vulva, and abdomen is more frequently observed as a clinical sign of affected pigs in Europe.

Hence, the European veterinary community has given various names to this syndrome: new pig disease, blue ear disease, porcine epidemic abortion and respiratory syndrome (PEARS), and the official names porcine reproductive and respiratory syndrome (PRRS). At a meeting in April 1991 European Community Veterinarians agreed on the name of “Porcine Reproductive and Respiratory Syndrome”-PRRS. In 1992, the ‘First International Symposium on PRRS’ in Minnesota adopted this term. Clinical manifestations are characterized by anorexia and respiratory distress in pigs of all ages; high mortality in neonatal and weaned pigs; poor conception in breeding herds; and

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reproductive disorders such as late-term abortions, premature farrowings, stillborns, mummifications, and weak live born pigs [5, 30]. PRRS virus (PRRSV), the causative agent of PRRS, is a single-stranded, positive-sense, enveloped RNA virus [1]. It belongs to the Arteriviridaefamily that also includes the equine arteritis virus (EAV), the lactate dehydrogenase-elevating virus (LDV) and the simian hemorrhagic fever virus (SHFV) [2, 19, 20]. The genome of PRRSV is about 15 kb in length and identified eight overlapping open reading frames (ORFs). ORFs 1a and 1b, which are expressed from genomic RNA, occupy more than two-thirds of the genome and encode the viral RNA polymerase [20]. Six putative structural proteins have been identified and assigned to distinct smaller ORFs. ORFs 2 to 7, respectively, encoded three major structural proteins, a 25 kDa envelope glycoprotein (GP5), an 18-19 kDa unglycosylated membrane protein (M), and a 15 kDa nucleocapsid (N) protein. Also, the translation products of ORFs 2, 3 and 4, with respective apparent molecular masses of 30, 45 and 31 kDa, have characteristics of membrane-associated glycoproteins [16, 21]. Antigenic and subsequent genetic analyses of PRRS viruses isolated from North America and Europe have revealed clear differences between viruses originating on the two continents [3, 7, 9, 13, 15-18, 22-24, 29]. PRRS virus is divided into two types, North American and European, genetically. According to data of the National Veterinary Research and Quarantine Service (NVRQS) in Korea (Table 5), there have been many importations of breeding pigs in Europe. The fact became a basis to make an estimate that there is an European PRRSV in Korea by inflow of European breeding pigs, and in the work we have detected the European PRRSV in Korean pig farms using polymerase chain reaction (PCR). And serologically, it also confirmed that there is not only North American PRRSV antibody but also a European PRRSV antibody.

Materials and Methods

Cells

A permissive clone (MARC-145) [8] derived from an African green monkey kidney (MA-104) cell line was used for virus propagation and the IFA test. The MARC-145 cells were propagated in growth medium (GM) was Dulbecco's modified eagle's medium (DMEM, Gibco-BRL, USA) supplemented with 10% fetal bovine serum (FBS), 1% antibiotics and antimycotics (Gibco-BRL, USA) was used for the propagation of cells. Maintenance medium (MM) consists of DMEM with 2% FBS, 1% antibiotics and antimycotics, and the cells were kept at 37°C in a humidified atmosphere containing 5% CO_2. The cells were obtained from NVRQS in Korea.

Viruses

North American type of the PRRSV (PL96-1) and EU prototype Leystad strains were used for serological assay. To isolate field viruses the lung tissues obtained from aborted piglets in Korea pig farms were homogenized with phosphate buffered saline (PBS). PRRS virus infections were represented in the contents of porcine alveolar macrophages by reverse transcriptase polymerase chain reaction (RT-PCR). The homogenized lung tissues were centrifuged and the resulting supernatants were passed through a 0.2 μm membrane filter to remove contaminated bacteria. The filtered sample was used as a virus stock for cell culture inoculation.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Common PCR primers were designed on the basis of ORF1b sequence. Type-specific (North America; NA and European; EU genotype) and type-common primers for multiplex or nested multiplex PCR (Table 1) were designed based on the sequence data of ORF 1b from two NA strains of PRRS virus: Minnesota MN-1b [9, 31] and Quebec LHVA-93-3 [14] isolates [4]. Viral RNA was extracted by acid guanidium thiocyanate phenol-chloroform method of Chomezynski and Sacchi. Mixture for the RT-PCR was prepared by following previously described protocols [4]. The primers predicted the amplification of 186 bp product for EU genotype and a 107 bp product for NA genotype (Table 1), respectively. A total of 21 PRRS virus strains and isolates propagated in cell culture were tested in the multiplex PCR assay. The multiplex PCR assay produced prominent DNA products for different PRRS viruses with titers that ranged from 1×10^4 to 3×10^5 $TCID_{50}$/100 μl. The limit of detection for the assay using RNA extracted from 10-fold dilutions of the MN-1b strain was 1×10^3 $TCID_{50}$ [4].

Sequencing and genetic analysis

The PCR products of 186 bp (EU genotype) were

Table 1. Oligonucleotide primers for PCR amplification and typing of PRRS viruses

Primer type and sequence (5' to 3')	Position in genome[a]	Size of PCR product (bp)	Genotype
Multiplex (or nested multiplex)			
U1 GTATGAACTTGCAGGATG	8634-8651	186	European
D1 GCCGACAATACCATGTGCTG	8800-8819		
U2 GGCGCAGTGACTAAGAGA	8713-8730	107	North American
D2 GTAACTGAACACCATATGCTG	8799-8819		
External for nested PCR			
EU CCTCCTGTATGAACTTGC	8628-8645	255	Common
ED AGGTCCTCGAACTTGAGCTG	8863-8882		

[a]Nucleotide positions are numbered according to the sequence of LV [20].

purified for sequencing from agarose gel and purified using the GENECLEAN KIT (Bio 101, USA) according to the manufacturer's recommendations. Sequencing was carried out by using Dye Terminator (ABI type Seq.) sequencing type by automated sequencing analyzer (Bionex, Korea). Sequencing data was analyzed using computer software program Align, Basic Local Alignment Search Tool (BLAST, National Center for Biotechnology Information, USA). Comparisons of 154 bp nucleotides were carried out using the DNASIS-DB (Hitachi Software Engineering Co., Ltd. DNASIS-DB for Window version 1.1). Information obtained by sequence analysis was used for comparing with other strains.

Serological tests by indirect fluorescence assay (IFA)

Antibody responses to the different strains were analyzed by IFA test as previously described [12]. Antibodies specific to NA (PL96-1) and EU (LELY STAD) genotype PRRSV were compared in serum samples collected from Korean swine herds. IFA test was carried out by following previously described protocol [25].

Results

RT-PCR

Detection of European type of PRRSV from the lung

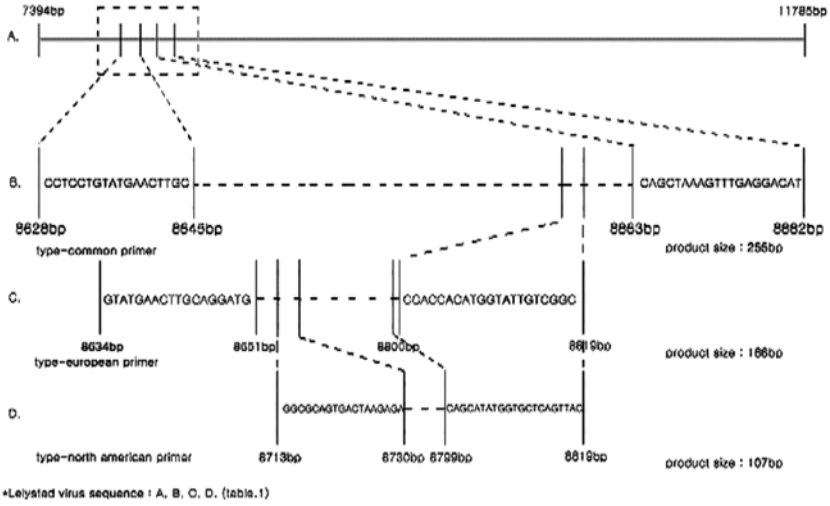

Fig. 1. Schematic diagram represent ORF1b of PRRS virus. Each fragment represents position of the primer and expected size of the PCR product.

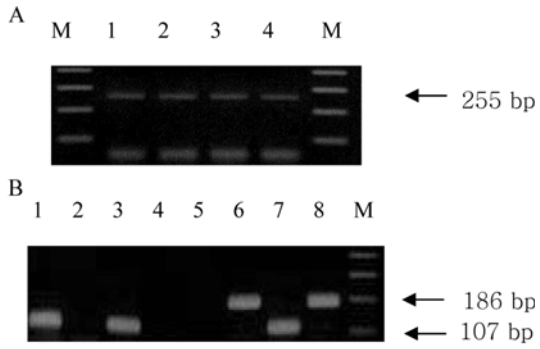

Fig. 2. Electrophoresis of RT-PCR for PRRS virus typing.
A.
Lane M: 100 bp DNA ladder
Lane 1: VR2332 (NA)
Lane 2: PL96-1 (NA)
Lane 3: LELYSTAD (EU)
Lane 4: KU-05
B.
Lane M: 100 bp DNA ladder
Lane 1, 2 : VR2332 (NA)
Lane 3, 4: PL96-1 (NA)
Lane 5, 6: LELYSTAD (EU)
Lane 7, 8: KU-05

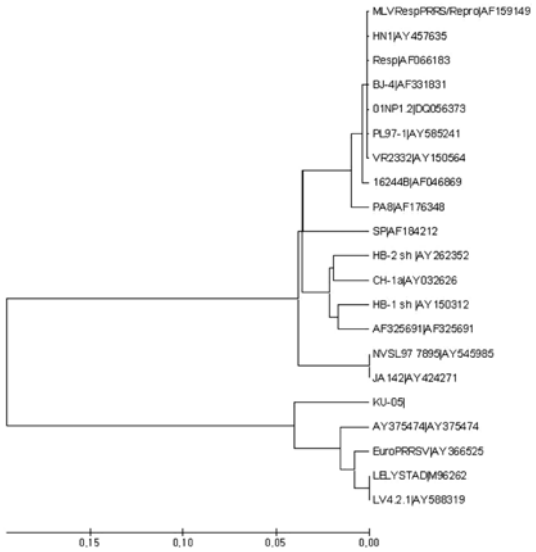

Fig. 4. Phylogenetic analysis of the nucleotide sequence of a portion of the polymerase gene (ORF1b) of PRRSV using computer programs (Molecular Evolutionary Genetics Analysis, version 3.0; MEGA3 1993~2005).

tissues obtained aborted piglets in Korean swine farms was performed by RT-PCR (Fig. 2). The nucleotide sequence of a portion of the polymerase gene (ORF1b) of PRRS virus field samples was amplified RT-PCR using a sets of type-common, NA and EU primer specific to PRRS virus ORF1b (Table 1). Primers are indicated at the each position as shown in Fig. 1. ORF1b was amplified by using the type specific primers. PCR products were electrophoresed on 2.5% agarose gel. As shown in Fig. 2, this result indicates the presence of EU genotype PRRSV as well as NA type virus.

Sequence analysis

The sequence of RT-PCR products specific to EU type (Fig. 2) was compared with other known ORF1b sequence (Fig. 1) and phylogenetic analysis was made by comparison with published data (Fig. 3, 4). Sequences of other known PRRS virus were downloaded from GenBank. Sequence containing upstream and downstream of the ORF1b showed variable homology among PRRS viruses (Table 2). As shown in Table 2 PRRSV strains similar to EU genotypes were genetically unique to NA strains of the PRRSV. Also there are minor changes between field strains similar to EU genotypes.

Serological tests by indirect fluorescence assay (IFA)

Serum samples were tested for PRRS virus specific antibody responses using indirect fluorescence assay (IFA) test. IFA was carried out for initial detection of European genotype PRRSV antibody. NA and EU

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KU-05          1  TGTATGAACTTGCAGGATGTGAAGAGTACCTGCCTAGTTATGTACTCAACTGTTGCCATG  60
                  ||||||||||||||||||||||||||||||||||| |||||||| ||||| || || ||||||||
EuroPRRSV   8592  TGTATGAACTTGCAGGATGTGAAGAGTACTTGCCTAGCTATGTGCTTAATTGCTGCCATG  8651

KU-05         61  ACCTTGTGGCAACGCAGGATGGTGCCTTCACAAAACGTGGTGGTCTGTCGTCTGGGGACC  120
                  |||||||||||||| |||||||||||||||||||||||||| ||||| |||||||| ||||||||
EuroPRRSV   8652  ACCTTGTGGCAACACAGGATGGTGCCTTCACAAAACGCGGTGGCCTGTCGTCCGGGGACC  8711

KU-05        121  CAGTCACCAGTGTGTCCAACACCGTGTATTCACT  154
                  | |||||||||||||||||||||| ||||||||
EuroPRRSV   8712  CCGTCACCAGTGTGTCCAACACCGTATATTCACT  8745
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Fig. 3. Nucleotide sequence analysis of the ORF1b gene of PRRSV indicates that KU-05 has high homology to an European strain.

Table 2. Comparison of PRRSV nucleotide homology using RT-PCR products. KU-05 was compared with other PRRSV strains

Strain	Homology (%)	Genotype	Accession No.
Lelystad	93.0	EU	AY150564
LV4.2.1	93	EU	AY588319
EuroPRRSV	92.5	EU	AY366525
AY375474	91.9	EU	AY375474
NVSL 97-7895	72.2	NA	AY545985
JA142	72.2	NA	AY424271
PA8	71.6	NA	AF176348
CH-1a	71	NA	AY032626
AF325691	70.5	NA	AF325691
HB-1(sh)	69.9	NA	AY150312
HB-2(sh)	69.3	NA	AY262352
16244B	71.6	NA	AF046869
01NP1.2	71.6	NA	DQ056373
PL97-1	71.6	NA	AY585241
VR2332	71.6	NA	AY150564
Resp	71.6	NA	AF066183
MLVRespPRRS/Repro	71.6	NA	AF159149
HN1	71	NA	AY457635
BJ-4	71	NA	AF331831
SP	69.3	NA	AF184212

NA: North American type, EU: European type, *PL97-1 strain [6].

Table 3. Serological tests result by Immunofluorescence assay against two different genotypes of PRRSV strains

	Antibody test against two different PRRSV stains								Total
	NA	EU	NA	EU	NA	EU	NA	EU	
	+	+	+	–	–	+	–	–	
No.	63		27		14		11		115
%	**54.7**		23.4		**12.1**		9.5		100

Table 4. Geographical distribution of type specific antibodies to PRRSV

Region	Antibody test against different PRRSV strains								Total
	NA	EU	NA	EU	NA	EU	NA	EU	
	+	+	+	–	–	+	–	–	
GyeongGi	30		11		9		5		55
Chungchong	8		5		·		2		15
Jeonla	9		4		2		·		15
Gyeongsang	4		2		3		1		10
Total	51		22		14		8		95

Table 5. The data of pigs imported in Korea (1996.01~2005.09)

	NA		EU		Asia	OZ
Country	USA	England	Ireland	Finland	Japan	Australia
Number of pigs	4763	3445	60	58	75	86
Country	Canada	Denmark	Sweden		Vietnam	
Number of pigs	5874	456	145		4	

genotype PRRSV antibody was compared using serum of Korean swine herd. Serological analysis of serum samples showed presence of specific antibodies to NA and EU type of PRRSV. Large numbers of pigs showed both NA and EU specific antibodies (Table 3, 4) which indicate that two different genotypes are circulating in the same herd. Results were analyzed based on geographical distribution (Table 4), single or double infection in the same samples (Table 3). Table 5 shows importation of pigs form foreign countries include European origin.

Discussion

PRRS virus is a very important factor causing economic loss in swine industry. Typically, it causes infertility, stillbirth, born weakness in breed herd, and it also causes severe PMWS (postweaning multisystemic wasting syndrome) signs complicated with porcine circovirus type2 infection. So the control of PRRS virus infection is the main issue for a long period. PRRSV infection in pig population is continuing problems in Korean swine industry [6]. Respiratory distress and reproductive failures caused by PRRSV has been common complaint by pig producers. Fortunately PRRSV vaccine has been purchased from North America strain (VR2332). PRRS virus is categorized into two types, North American and European [21, 22]. There is a little genetic homology between North American and European strain [4]. So there is a big possibility to be European virus in Korea, because Korea imports thousands of pigs from Europe for breeding stocks (Data of the NVRQS) in Korea. There has been no report and paper about European PRRSV in Korea. We have detected the European PRRSV in Korean pig herds using PCR and antibodies raised against European type of PRRSV by IFA test [25]. Genetic determination of Korean isolate KU-05 strain showed high homology of ORF1b region of the European prototype Lelystad strain of PRRSV. And serologically, it is also confirmed that there is not only North American PRRSV antibody but also antibody to European PRRSV in Korean pig farms. This results are not unexpected because of large number of breeding stocks were imported over long period of time during PRRSV endemic in European countries.

Conclusion

These results indicate that the European type of PRRSV is circulating in Korean swine herds and need to further investigation for the significance of the virus in pig population. So there is the need to introduce a proper vaccine (EU strains) to control the prevalence of PRRSV in Korea.

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