수의학석사학위논문

Molecular detection and genetic analysis of porcine bocavirus in Korean domestic swine herd

국내 돼지에서의 bocavirus 검출 및 분자유전학적 분석

2014년 2월

서울대학교 대학원

수의학과 수의미생물학 전공

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A dissertation submitted to the faculty of the Graduate School of Seoul National University in partial fulfillment of the requirement for the degree of Master in Veterinary Microbiology

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Abstract

Molecular detection and genetic analysis of porcine bocavirus in Korean domestic swine herd

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Several porcine bocaviruses have been detected worldwide, and this report is the first to detect this virus from Korean domestic swine herd. Until now, three major genetic groups of porcine bocavirus (PBoV) were identified, named PBoV1, PBoV2 and PBoV3. This virus is known to show worldwide distribution including Europe, Africa, North America and Asia. However, there was no report yet about the presence of PBoV in Korean swine farms. The prevalence of porcine bocavirus was identified with various samples, including serum, tissue, feces and saliva from healthy or diseased pigs. In case of saliva, this is the first finding to prove the presence of PBoV in saliva of swine.

Through PCR assay for each three groups of PBoV, I revealed the fact that all of PBoV showed higher infection rates in clinically diseased animals, especially for PBoV 1 (16.8%) and PBoV 3 (10.9%). This study also suggested that all porcine

bocavirus groups primarily infected weaned piglets through the survey of positive rates using sera from different age group pigs.

The genetic analysis was performed with the ORF3 gene, which is a unique gene to bocavirus to analyze the genetic relationship between the Korean strains and reference strains from other countries. The primer sets were designed for this study, and we got 14 different genomic sequences of Korean PBoV NP1 protein coding gene. As comparing these unique sequences of Korean strains with other dated sequences in GenBank that originated from other countries, we found that most Korean PBoV sequences showed high similarity with other typical strains with the one exception of 6133 (PBoV2) strain. The strain did not have high similarities (85.1%~87.7%) to any other sequences of PBoV, and it seemed a variant belonged to PBoV 2.

This study characterized the current status of PBoV infection and the molecular epidemiologic information in Korean swine farms. In addition, several interesting facts of this new emerging virus were revealed including viral shedding through saliva.

Keywords: Porcine bocavirus, clinical relationship, Age difference, ORF3

Student ID: 2012-21551

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INTRODUCTION

Bocavirues belong to the *Parvoviridae* family, exibit linear, single-stranded genomic DNA, and uniquely possess an additional open reading frame (ORF), ORF3. This gene encodes non-structural protein 1 (NP1), which is known for its critical roles in viral surviva (Sun *et al*., 2009; Sun *et al*., 2013). In 2009, the first novel porcine bocavirus (PBoV) was identified, termed porcine boca-like virus (PBo-like V), in lymph nodes from a pig that afflicted with the postweaning multisystemic wasting syndrome (PMWS) in Sweden (Blomström *et al*., 2009). Since that finding, several porcine bocavirus sequences have been revealed from various countries. In China, the infection rates of PBoV 1, PBoV 2 and PBoV 3 were 28.9%, 19.3% and 39.7%, respectively (Zeng *et al*., 2011). Moreover, 2.3%, 7.4% and 2.8% of fecal samples were positive for PBoV 1, PBoV 2 and PBoV 3, respectively, in Hungary (Cságola *et al*., 2012).

In this study, we reported the first detection of PBoV in a Korean domestic swine farm and analyzed the genetic relationships between Korean PBoV and bocaviruses from other countries. To assess the PBoV prevalence by group, we adopted the classification proposed by Xiao *et al*. (2013), and thus the PBoV was classified into three groups (PBoV 1, PBoV 2 and PBoV 3) (Xiao *et al*., 2013). A large-scaled nationwide survey was conducted to identify the PBoV prevalence in different age groups of pigs using serum samples. In addition, several swine samples, including tissue, feces and saliva, were surveyed for the prevalence of PBoV in Korean pigs. A genetic analysis of the Korean strain was

performed using the ORF3 genomic sequence, which is a unique feature of bocaviruses. The sequence of this gene is dated in GenBank with the highest number among three different ORFs (NS1, NP1 and VP1/2). Moreover, this is the shortest ORF in PBoV (657 nt ~ 692 nt long). For this reason, the ORF3 was used for genetic comparison. From the comparison of ORF3 sequences, we could monitor the existence of the bocavirus variant in Korean swine farms.

MATERIALS AND METHODS

Sample collection

Nine hundred and twenty porcine samples, including serum, feces, tissue and saliva, were collected between July 2011 and January 2013 from 142 farms in nine South Korean provinces. The samples collected for this study were classified into two groups, normal and sick (cough, inappetence, lethargy, fever and wasting) pigs, and most farms selected for this study sent us an even number of samples collected from pigs of each age group. Various pig tissues were pooled into one tube, and these pooled tissue samples included main organs, such as the lymph nodes, tonsils, heart, lungs, liver, spleen and kidneys, except intestinal organs. Only the lung and lymph node tissues were separated as targeting organs, due to the difficulty of sample collection. All of these tissue samples were taken from alive or dead pigs showing the most severe symptoms. The saliva samples were collected using cotton rope that was hung in a pen. One cotton rope per pen was positioned to interact with pigs, and a pen only included pigs of the same age. After exposure, the ropes were inserted in a resealable plastic bag to extract oral fluids.

Molecular detection and statistical analysis

The DNA was extracted using the Viral DNA/RNA extraction Kit (iNtRON BIOTECHNOLOGY, Gyeonggi, South Korea, Inc.) and was

immediately used for amplification or stored at -20℃. The samples were tested with three different primer sets targeting each PBoV group (PBoV 1, PBoV 2 and PBoV 3). PBoV 1 was detected with PCR using the primers designed by Zhai *et al*. (2010), and PBoV 2 and PBoV 3 were detected with the primer pairs from the study of Cságola *et al.* (2012). The PCR reaction was performed using the i-StarMaster Mix PCR kit (iNtRON BIOTECHNOLOGY, Gyeonggi, South Korea, Inc.), and the PCR cycle conditions taken from the primer set manuscript comments from Zhai *et al*. (2010) and Cságola *et al.* (2012). The statistical analyses were performed using Pearson's chi-squared test in PASW statistics 17.0, and a p value of <0.05 was considered statistically significant.

Sequencing and genetic analysis

To identify the sequence of the NP1 protein coding gene of PBoV, a PCR reaction was conducted using the primer sets for sequencing PBoV 1, which were designed by Zheng *et al.*(2011).

PBoV 2 (PBoV2NPF:5'-GCCGAGAGTCACCTTCTACG-3',
PBoV2NPR:5'-CCGTTCTCTATCGGGTTGAA -3') and
PBoV3 (PBoV3NPF: 5'-AGATAGGAAACACAAGGATAGC-3',
PBoV 3NPR:5'-TCGTTGAGCGCAGGCGCAATTT-3')

were amplified with specific primers that were designed and optimized in this study. The DNA fragments were sequenced (Macrogen, Seoul, South Korea) in triplicate to ensure accuracy. The sequences were aligned with other PBoV ORF3 sequences dated in GenBank using clustalW version 1.83

(http://clustalw.ddbj.nig.ac.jp/top-e.html) and the BioEdit Sequence Alignment Editor 7.0.4 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Phylogenetic and genomic comparison studies were conducted using MEGA5.0 and MegAlign in DNASTAR version 5.0, respectively.

RESULTS

Prevalence of PBoV

PBoV was present in Korean domestic swine herds with a high detection rate. Out of 920 swine samples, 321 samples (34.9%) possessed more than one PBoV group. We demonstrated that all three PBoV groups were present in Korean swine populations (12.2%, 17.4% and 17.8% for PBoV 1, PBoV 2 and PBoV 3, respectively), and the PBoV 3 was most prevalent in Korea. Comparing the prevalence of serum from normal pigs and clinically disordered ones, we observed significant infection rate in diseased pigs with PBoV1 and PBoV3 ($p<0.05$, χ^2 test). The PBoV prevalence in the serum of normal pigs varied with age, especially in the weaned group, which exhibited a significant infection rate compared to other age groups. Almost half of the weaned pigs were infected with one or more PBoV groups (44.5%), as assessed using serum. Considering the group results, the positive rate was 18.1%, 13.6% and 12.7% for the PBoV 1, PBoV 2 and PBoV 3, respectively, in weaned pigs, and these infection rates were significantly higher than the rest of the pig age groups ($p<0.05$, χ^2 test) [Table 1].

Table 1. Analysis of PBoV infection status in groups using various samples and comparing the differences in the prevalence between age groups in serum samples

Clinical status	Type of Samples		No. of positive (positive rate)			Total (%)
			PBoV 1 (%)	PBoV 2 (%)	PBoV 3 (%)	/ co-infection[b]
Normal (*n*=679)	Serum[a]					
		Gilt (*n*=47)	0	0	0	0
		Sow (*n*=72)	1 (1.4)	2 (2.8)	0	3 (4.2)
		Suckling (*n*=40)	0	4 (10.0)	0	4 (10.0)
		Weaned (*n*=110)	20 (18.1)	15 (13.6)	14 (12.7)	49 (44.5) / 5
		Grower (*n*=87)	5 (5.7)	6 (6.9)	2 (2.3)	13 (14.9)
		Finisher (*n*=101)	1 (1.0)	3 (3.0)	0	4 (4.0)
		Subtotal (*n*=457)	27(5.9)	30 (6.6)	17 (3.7)	68 (14.9) / 5
	Feces (*n*=180)		41 (22.8)	80 (44.4)	93 (51.7)	131 (72.8) / 83
	Saliva (*n*=42)		13 (31.0)	14 (33.3)	18 (42.9)	31 (73.9) / 11
Clinically disordered and deceased (*n*=351)	Serum (*n*=119)		20 (16.8)	14 (11.8)	13 (10.9)	46 (38.7) / 1
	Pooled tissue (*n*=69)		5 (7.2)	14 (20.3)	18 (26.1)	32 (46.4) / 5
	Lung (*n*=34)		0	1 (2.9)	1 (2.9)	2 (5.9)
	Lymph node (*n*=19)		6 (31.6)	7 (36.8)	4 (21.1%)	11 (57.9) / 5
Grand total (*n*= 920)			112 (12.2)	160 (17.4)	164 (17.8)	321 (34.9) / 110

[a]Serum samples were sorted into six groups: female (gilt and sow), piglet (suckling, <30 days; weaned, 30≤-<60 days; grower, 60≤-<90 days; and finisher, 90 days≤)

[b]When we detected more than one PBoV group from a sample, the sample was tentatively considered as co-infected.

Genetic and phylogenetic analysis

In this study, we identified the genomic sequences of fourteen different NP1 protein coding PBoV sequences from various type of samples: four PBoV 1, four PBoV 2 and six PBoV 3 sequences. The nucleotide and deduced amino acid sequences of the Korean strains were compared to each other [Table 2]. In addition, these Korean strains were compared with representative strains of each group that were identified from other countries [Table 3]. In the comparisons of nucleotide and deduced amino acids, strains belonged to PBoV 1 were highly similar, more than 98.1%~99.2% to the reference strain FJ872544 which is the first isolate, PBo-like V. In contrast to PBoV 1 strains, the Korean strains that belonged to PBoV 2, 6133/2011 only displayed 85.1%~87.7% identity in the nucleotide sequences and 77.5%~81.8% in the deduced amino acid sequences compared to the reference strains HM053693 and HM053694 [Table 3].

Table 2. Summary of nucleotide and deduced amino acid similarity in ORF3 gene between the Korean porcine bocavirus each other identified in this study

PBoV group	Virus / Isolation year	Isolated sample type / Health status	6141	5276	1628	1972	6133	5628	8516	3584	6132	6514	3599	3070	3545	3552
PBoV 1	6141 / 2011	Serum / Diseased	***	**98.2**	**98.6**	**98.7**	50.9	53.2	53.4	53.6	44.3	43.9	44.3	44.4	44.7	44.6
	5276 / 2011	Feces / Normal	**95**	***	**97.9**	**98.7**	50.2	52.6	52.9	53	44.2	43.7	44.2	44.3	44.6	44.6
	1628 / 2012	Pooled tissue / Diseased	**100**	**95**	***	**98.5**	50.4	52.7	53	53.2	44.3	43.9	44.3	44.4	44.9	44.7
	1972 / 2012	Saliva / Normal	**98.3**	**98.3**	**98.3**	***	50.6	53	53.3	53.4	44.7	44.3	44.7	44.9	44.9	44.7
PBoV 2	6133 / 2011	Serum / Normal	30	31.7	30	30	***	84.2	84.5	86.4	47.8	47.4	47	47.1	45.3	45.6
	5628 / 2011	Feces / Normal	23.3	33.7	23.3	32.3	68.3	***	99.7	**91.3**	49.1	48.1	48.4	48.8	48.4	48.7
	8516 / 2012	Lymph node / Diseased	23.3	25	23.3	23.3	68.3	100	***	**91.6**	49.1	48.1	48.4	48.8	48.4	48.7
	3584 / 2012	Saliva / Normal	25	32	25	30.7	75	**93.3**	**91.7**	***	48.2	47.7	47.8	48.1	46.4	46.8
PBoV 3	6132 / 2011	Serum / Normal	36.7	36	36.7	35.7	38.3	31.3	41.7	30	***	**98.6**	**98**	**97.7**	83.4	82.8
	6514 / 2011	Feces / Normal	51.1	53.3	51.1	51.1	40	42.2	42.2	37.8	**95.6**	***	**98.3**	**97.5**	82.8	82.3
	3599 / 2012	Feces / Normal	40	36.7	40	36.3	35	30.3	38.3	29	**98.5**	**100**	***	**96.9**	82.6	82
	3707 / 2012	Pooled tissue / Diseased	51.1	53.3	51.1	51.1	40	42.2	42.2	37.8	**95.6**	**100**	**100**	***	82	81.7
	3545 / 2012	Lymph node / Diseased	33.3	35	33.3	34.3	28.3	28	33.3	26.3	81.7	77.8	80.8	77.8	***	97.7
	3552 / 2012	Saliva / Normal	48.9	48.9	48.9	48.9	37.8	37.8	37.8	33.3	84.4	80	80	80	93.3	***

*Upper triangle for nucleotide similarities and lower triangles for deduced amino acid sequence similarities.

**Bold text represents a higher than 90% similarity.

Table 3. Similarities of the nucleotide and deduced amino acid sequences (Nucleotide (%) / deduced amino acid (%)) of the complete ORF3 in the PBoV genes.

Korean strain/ Year (Accession number)	PBoV 1	PBoV 2		PBoV 3					
	FJ872544	HM053693	HM053694	JF429834	JF429835	JF512472	JF512473	JN681175	JN831651
6141/2011 (KF425336)	**99.2/99.5**	51.3/38.4	51.1/39.7	40.5/29.2	41.3/29.5	40.8/28.8	40.7/29.2	39.3/29.2	41.9/31.5
5276/2011 (KF728246)	**98.1/97.5**	53.4/31.0	53.5/32.0	45.1/48.9	45.1/26.0	45.5/48.9	44.4/26	43.4/43.3	45.5/26.0
1628/2012 (KF728243)	**98.2/97.5**	53.7/25.8	53.8/27.5	45.2/46.7	45.2/30.8	45.6/46.7	44.2/31.7	43.4/40	45.6/29.2
1972/2012 (KF728244)	**98.6/98.3**	54.0/31.0	54.1/32.0	45.5/46.7	45.5/24.3	45.9/46.7	44.8/25.0	43.8/40.0	45.5/24.7
6133/2011 (KF425335)	46.5/35.5	85.1/77.5	87.7/81.8	44.3/35.1	45.3/33.9	43.3/33.3	45.0/33.2	43.1/32.5	43.0/32.0
5628/2011 (KF728247)	53.3/25.8	**91.0/91.3**	**91.8/93.7**	48.1/46.7	47.7/32.0	47.3/46.7	47.8/29.3	47.3/33.3	48.7/29.3
8516/2012 (KF728248)	53.5/25.8	**91.1**/87.5	**92.1/93.3**	48.1/46.7	47.7/33.3	47.3/46.7	47.8/26.7	47.3/33.3	48.7/30.0
3584/2012 (KF728245)	54.2/25.0	**93.9**/88.3	**93.6/93.3**	48.0/42.2	47.6/30.8	46.9/42.2	47.7/24.2	47.7/26.7	47.1/27.5
6132/2011 (KF425334)	39.8/28.2	43.6/37.0	44.6/36.6	**99.0/98.7**	**98.7/98.7**	**96.5/96.0**	83.8/79.6	79.9/73.1	82.8/76.2
3599/2012 (KF425332)	39.4/27.8	43.6/37.0	44.1/36.6	**98.4/97.8**	**98.7/97.4**	**96.3/95.2**	83.0/78.3	79.3/72.2	82.2/75.8
6514/2012 (KF425337)	39.8/27.8	44.1/37.4	44.8/37.0	**97.5/97.8**	**98.5/98.2**	**95.4/95.2**	83.5/78.8	80.8/73.6	81.9/75.8
3707/2012 (KF425333)	39.9/27.8	43.6/36.1	44.6/35.7	**97.8/97.4**	**97.1/96.9**	**96.9/97.4**	83.1/79.2	78.6/71.8	81.4/74.4
3545/2012 (KF425330)	40.7/30.7	43.6/33.8	43.3/33.8	83.0/75.6	83.5/75.9	83.1/74.2	87.2/82.3	78.7/71.1	**98.4/96.9**
3552/2012 (KF425331)	39.8/30.1	43.8/33.6	43.5/34.1	82.6/76.1	83.0/75.8	82.4/73.9	86.6/82.3	77.7/70.4	**98.1/98.2**

*Korean isolates were compared with other complete sequences representative of each PBoV group. To prevent confusing between strain names and PBoV group names, we used accession numbers of each strain.

**Bold text represents a higher than 90% similarity

Moreover, in the phylogenetic tree, this strain yielded a separated branch from the main branch of PBoV 2 and displayed a close relationship with bocaviruses isolated from other species [Figure 1]. With PBoV 3, we revealed six sequences of the ORF3 gene. Four of them (6132/2011, 3599/2012, 6514/2012 and 3707/2012) were highly similar to the reference strains JF429834 and JF512472. The rest of sequences, 3545/2012 and 3552/2012, were belonged to PBoV3 also, and showed the highest similarities with JN831651 that was identified most recently (Xiao *et al.*, 2013). The PBoV 3 of porcine bocavirus possesses several subgroups, but in this study, we only identified some of them (Xiao *et al.*, 2013; Yang *et al.*, 2012). It is high possibility that other different strains that would belong to other subgroups can be detected; therefore, further study is necessary to reveal the genetic variety of the Korean strains.

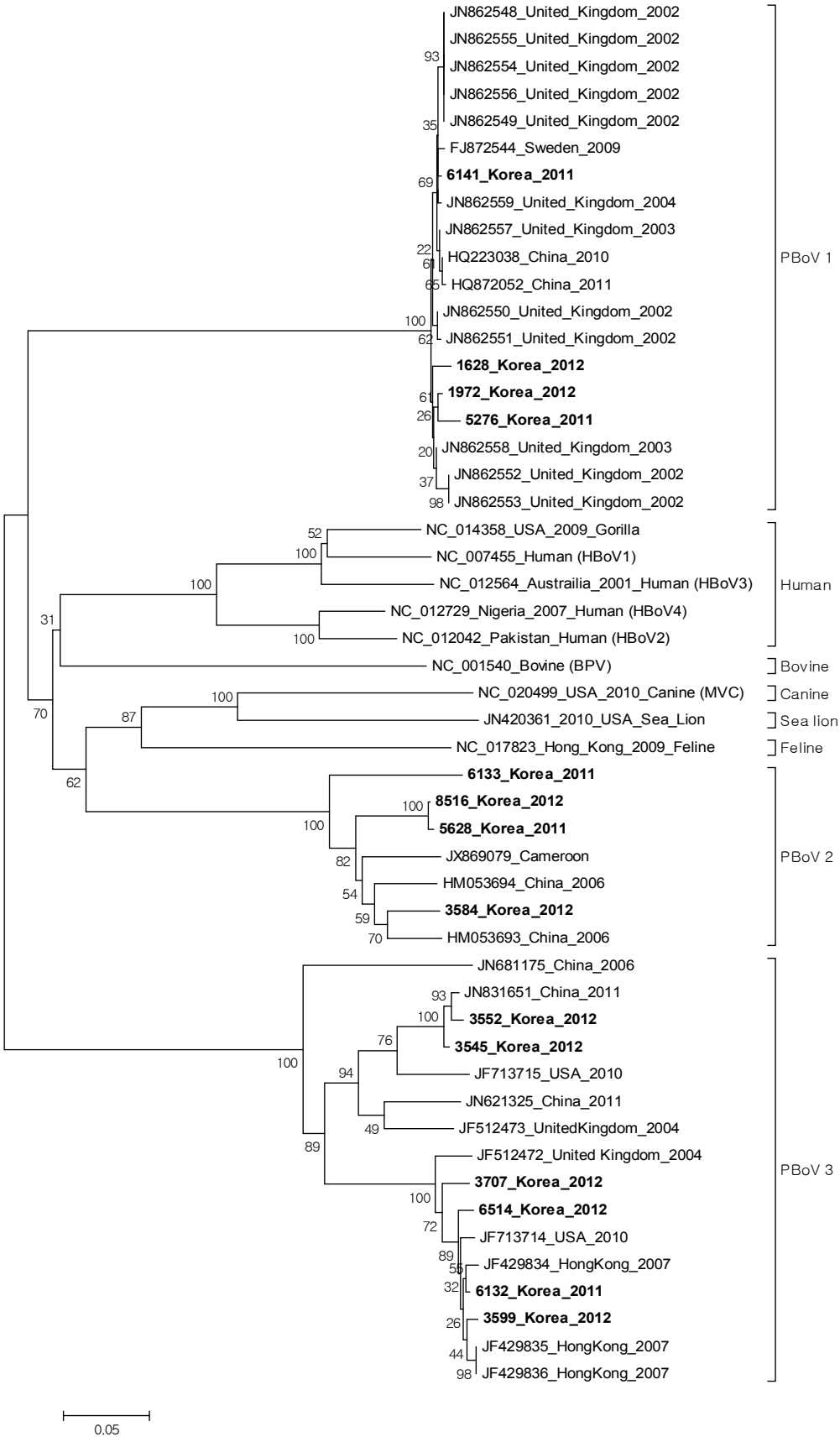
JN862548_United_Kingdom_2002
JN862555_United_Kingdom_2002
JN862554_United_Kingdom_2002
JN862556_United_Kingdom_2002
JN862549_United_Kingdom_2002
FJ872544_Sweden_2009
6141_Korea_2011
JN862559_United_Kingdom_2004
JN862557_United_Kingdom_2003
HQ223038_China_2010
HQ872052_China_2011
JN862550_United_Kingdom_2002
JN862551_United_Kingdom_2002
1628_Korea_2012
1972_Korea_2012
5276_Korea_2011
JN862558_United_Kingdom_2003
JN862552_United_Kingdom_2002
JN862553_United_Kingdom_2002
PBoV 1
NC_014358_USA_2009_Gorilla
NC_007455_Human (HBoV1)
NC_012564_Austrailia_2001_Human (HBoV3)
NC_012729_Nigeria_2007_Human (HBoV4)
NC_012042_Pakistan_Human (HBoV2)
Human
NC_001540_Bovine (BPV)
Bovine
NC_020499_USA_2010_Canine (MVC)
Canine
JN420361_2010_USA_Sea_Lion
Sea lion
NC_017823_Hong_Kong_2009_Feline
Feline
6133_Korea_2011
8516_Korea_2012
5628_Korea_2011
JX869079_Cameroon
HM053694_China_2006
3584_Korea_2012
HM053693_China_2006
PBoV 2
JN681175_China_2006
JN831651_China_2011
3552_Korea_2012
3545_Korea_2012
JF713715_USA_2010
JN621325_China_2011
JF512473_UnitedKingdom_2004
JF512472_United Kingdom_2004
3707_Korea_2012
6514_Korea_2012
JF713714_USA_2010
JF429834_HongKong_2007
6132_Korea_2011
3599_Korea_2012
JF429835_HongKong_2007
JF429836_HongKong_2007
PBoV 3
0.05

Figure 1. Evolutionary relationship of PBoV based on the complete ORF3 gene (The Korean strain sequences identified in this study are in bold). The phylogenetic relationship of the nucleotides (657 nt ~ 692 nt long) was inferred using the Neighbor-Joining method with 1,000 bootstrap replicates using the MEGA 5.0 software package. The bootstrap values were shown next to the nodes of the tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site (scale bar, 0.05 nucleotide substitutions per site).

DISCUSSION

In conclusion, this is the first description of the prevalence of PBoV in Korean swine herds and demonstrates the considerable prevalence of PBoV in Korea. Of the 920 samples, the mean positive rate was 34.9%, and the fecal sample result presented the highest prevalence among various porcine samples, 81% in total. Until now, the prevalence of PBoV has been reported in numerous countries and was variable in its geographical distribution. The positive rates were 28.9%, 19.3% and 39.7% for PBoV 1, PBoV 2 and PBoV 3, respectively in China (Zeng *et al*., 2011). In Europe, the positive rates were 9.14% (2006/2007) and 17.74% (2010/2011) of group 1 porcine bocavirus that infected wild boars in Romania (Cadar *et al*., 2011), and 2.3%, 7.4% and 2.8% of fecal samples were positive for PBoV 1, PBoV 2 and PBoV 3, respectively, in Hungary (Cságola *et al*., 2012). In Uganda on the African continent, PBoV 1 infected 2.1% of domestic pig serum (Blomström *et al*., 2012). This difference in the positive rate may due to the sampling time, sampling size and different raising environment such as climate, farm density and different raising systems (indoor/outdoor). In addition, the positive rates of the lung tissues from this study were 0%, 2.9% and 2.9% for PBoV1, PBoV2 and PBoV3, respectively, and this result was similar for the study in Hungary (2.6%, 2.6% and 0% for PBoV1, PBoV2 and PBoV3, respectively) (Cságola *et al*., 2012). In contrast, in China, the positive rate of PBoV1 from conventional PCR was 61.1% and 69.4% from real-time PCR, from diseased lung samples (Li *et al*., 2011). The possible reason for this

difference could be the limit sample numbers (this study employed 34 lungs; the Hungary study 38 lungs; the China study employed 36 lungs). Moreover, PBoV prevalence varied with age, geography and the season. These conditions may cause this considerable difference in the positive rate.

Our investigation indicated that the clinical samples displayed a higher infection rate for all PBoV groups, especially PBoV 1 and PBoV 3, which significantly prevailed. Several studies previously asserted that PBo-like V (PBoV 1) correlated with respiratory disease and/or PMWS (Blomström *et al*., 2009; Zhai *et al*., 2010). In the China study, the real-time PCR assay also revealed a significant difference between normal and sick pigs infected with PBoV1, which was observed in 16% of the serum of healthy pigs and 55.5% of the serum of diseased pigs (Li *et al*., 2011). However, except PBo-like V, a clinical relationship between of PBoV 2 and PBoV 3 was not identified. This study underscores the need for further analysis of the mechanisms that govern illness of pigs caused by other PBoV groups, especially PBoV 3.

Recent studies have revealed the presence of PBoV in various types of porcine samples, including feces, tissues and serum samples, but have not yet demonstrated its presence in saliva. In general, saliva is known as one of the major viral shedding routes, and the existence of HBoV in the saliva of children has already been revealed (Martin *et al*., 2009). This study demonstrated the existence of porcine bocavirus in saliva samples with the second highest positive rate after fecal samples, although the result should be interpreted cautiously as the saliva sample result represents the prevalence per pen, which may be an overestimate the actual

individual positive rate. To validate the fact that saliva is one of the viral shedding routes, further studies must be performed with oral-fluid samples taken directly from pigs. In this study, we used saliva samples collected from cotton rope, which could have been contaminated with feces and dirt from the farm.

The role of the NP1 protein of the porcine bocavirus has not been characterized, but the NP1 protein is known to play a critical role in viral replication in the case of MVC, which is the genetically closest species to PBoV (Yang *et al*., 2012). In this study, we identified 14 complete genomic ORF3 sequences that encode the NP1 proteins (Kapoor *et al*., 2010). Most of Korean isolate sequences were highly similar to the representative strains of the three groups from above. However, one isolate, 6133/2011 that belonged to PBoV 2 displayed a low similarity to the reference strains. The genomic diversity of PBoV 3 is well-established, and because of this feature, numerous novel porcine bocaviruses of PBoV 3 have been continuously discovered continuously (Susanna *et al*., 2011). Until today, however, PBoV2 isolates were extremely similar to each other and lacked a subgroup. The identification of the variant 6133/2011 suggests that there may be a new subgroup of PBoV 2 in Korean swine populations or a new variant that evolved currently. Identifying the remaining PBoV sequences (ORF1 and ORF2) of this variant is need for further elucidating these relationships. From the phylogenetic analysis, this variant also formed a distinct branch from the main PBoV 2 branch, and was closely related to the bocaviruses from other host species, including canine, sea lion, and human. As an emerging porcine virus, numerous distinct PBoV strains with variable genomic sequences are continuously being detected worldwide. Thus, it is possible

that many variants such as 6133/2012 exist but have yet to be identified. In spite of the potential danger of PBoV because of its high prevalence in pig populations and its wide range of genetic diversity, there remains a paucity of information about this virus (Zhai *et al*., 2010; Susanna *et al*., 2011). Therefore, further studies focusing on its epidemiology and genomic analysis must be pursued as well as the viral isolation and its characterization. In addition, the assertion that PBoV is the causative agent should be qualified with direct evidence.

REFERENCES

Blomström AL, Belak S, Fossum C, McKillen J, Allan G, Wallgren P, Berg M (2009) Detection of a novel porcine boca-like virus in the background of porcine circovirus type 2 induced postweaning multisystemic wasting syndrome. Virus Research 146:125-129

Blomström AL, Stahl K, Okurut AR, Masembe C, Berg M (2012) Genetic characterisation of a porcine bocavirus detected in domestic pigs in Uganda. Virus Genes

Brieu N, Guyon, Gael, Rodiere, Michel, Segondy, Michel, Foulongne, Vincent (ed) (2008) Human Bocavirus Infection in Children With Respiratory Tract Disease. Pediatric Infectious Disease Journal

Cadar D, Cságola A, Lorincz M, Tombacz K, Kiss T, Spinu M, Tuboly T (2011) Genetic detection and analysis of porcine bocavirus type 1 (PoBoV1) in European wild boar (Sus scrofa). Virus Genes 43:376-379

Carmichael LE SD, Hashimoto A (1991) Pathogenicity of minute virus of canines (MVC) for the canine fetus. The Cornell Veterinarian 81:151-171

Cságola A, Lorincz M, Cadar D, Tombacz K, Biksi I, Tuboly T (2012) Detection, prevalence and analysis of emerging porcine parvovirus infections. Archives of Virology 157:1003-1010

Durham PJ, Lax A, Johnson RH (1985) Pathological and virological studies of

experimental parvoviral enteritis in calves. Research of Veterinary Science 38:209-219

Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L (2010) Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. Journal of Infectious Diseases 201: 1633–1643.

Li B, Xiao S, Ma J, Liu Y, Mao L, Wen L, Mao A, Zhang X, Ni Y, Guo R, Zhou J, Yu Z, Lv L, Wang X, Fang L, Chen H, He K (2011) Development of a novel TaqMan-based real-time PCR assay for the detection of porcine boca-like virus (Pbo-likeV). Virology Journal 8:357

Martin ET, Taylor J, Kuypers J, Magaret A, Wald A, Zerr D, Englund JA (2009) Detection of bocavirus in saliva of children with and without respiratory illness. Journal of Clinical Microbiology 47:4131-4132

Sun B, Cai Y, Li Y, Li J, Liu K, Li Y, Yang Y (2013) The nonstructural protein NP1 of human bocavirus 1 induces cell cycle arrest and apoptosis in HeLa cells. Virology 440:75-83

Sun Y, Chen AY, Cheng F, Guan W, Johnson FB, Qiu J (2009) Molecular characterization of infectious clones of the minute virus of canines reveals unique features of bocaviruses. Journal of Virology 83:3956-3967

Susanna K. P. Lau, Patrick C. Y. Woo, Cyril C. Y. Yip, Kenneth S. M. Li, Clara T. Y. Fu, Yi Huang, Kwok-Hung Chan, Kwok-Yung Yuen (2011)

Co-existence of multiple strains of two novel porcine bocaviruses in the same pig, a previously undescribed phenomenon in members of the family *Parvoviridae*, and evidence for inter- and intra-host genetic diversity and recombination. The Journal of General Virology 92: 2047–2059

Xiao CT, Halbur PG, Opriessnig T (2013) Molecular evolutionary genetic analysis of emerging parvoviruses identified in pigs. Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases 16:369-376

Yang WZ, Yu JM, Li JS, Cheng WX, Huang CP, Duan ZJ (2012) Genome characterization of a novel porcine bocavirus. Archives of Virology 157:2125-2132

Zeng S, Wang D, Fang L, Ma J, Song T, Zhang R, Chen H, Xiao S (2011) Complete coding sequences and phylogenetic analysis of porcine bocavirus. The Journal of General Virology 92:784-788

Zhai S, Yue C, Wei Z, Long J, Ran D, Lin T, Deng Y, Huang L, Sun L, Zheng H, Gao F, Zheng H, Chen S, Yuan S (2010) High prevalence of a novel porcine bocavirus in weanling piglets with respiratory tract symptoms in China. Archives of Virology 155:1313-1317

국문초록

국내 돼지에서의 bocavirus 검출 및 분자유전학적 분석

최민경

수의미생물학전공

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돼지 bocavirus(PBoV)는 현재 세계 여러 나라에서 분자생물학적 방법을 통해 그 존재가 증명되고 있으며, 본 논문은 국내 돼지 농가의 PBoV 존재 여부 및 감염실태를 처음으로 조사한 연구이다. 지금까지 발견된 PBoV 는 크게 유전형을 바탕으로 세 그룹으로 분류되고 있으며, 각 그룹은 PBoV 1, PBoV 2, PBoV 3 라 불리고 있다. 본 바이러스는 앞서 언급한 바와 같이 전 세계적으로 널리 분포하는 것으로 알려져

있으며, 이미 유럽, 아프리카, 북미, 아시아 각지에서 PBoV 의 감염 사실이 규명되고 있으나 아직 국내에서는 PBoV 의 존재 여부를 증명하는 연구 결과가 발표된 적이 없다. 본 연구에서는 다양한 돼지 시료를 수집하여 각 그룹별 PBoV 의 양성률 및 분자역학적 특징을 분석하였고, 건강돈 및 질환돈으로부터 채취한 혈청, 조직, 분변, 타액을 실험에 사용하였다.

PCR 을 통해 세 그룹의 PBoV 양성률을 각각 조사하였고, 그 결과 PBoV 의 감염률이 돼지의 건강상태와 관련이 있음을 알 수 있었다. 특히 PBoV 1(16.8%) 과 PBoV 3 (10.9%) 의 경우 질환돈의 혈청내에서 훨씬 높은 빈도로 검출되었다. 또한 연령별 양성률 비교를 통해 본 바이러스가 그룹에 관계없이 이유기 자돈에 주로 감염됨을 알 수 있었다.

국내 감염 PBoV 의 유전적 특성을 분석하기 위해 PBoV 의 고유의 유전자인 ORF3 의 서열을 분석하였다. 해당 실험을 위해 디자인 된 primer 를 이용하여 14 종의 국내 돼지유래 PBoV ORF3 염기서열을 얻을 수 있었다. 분석결과 본 연구에서 밝혀진 대부분의 서열이 기존에 GenBank 에 보고되어 있는 다른나라의 PBoV 와 높은 유사성을 보임을 알 수 있었다. 그러나 6133 (PBoV2) strain 의 경우 많은 변이를

가지고 있어 어떠한 바이러스의 염기서열과도 유사도가 높지 않음을 발견하였다 (85.1%~87.7%).

본 연구는 PBoV 의 국내 감염실태를 조사함과 동시에, 분자역학적 측면에서 특징을 연구하였다. 또한 아직 밝혀지지 않았던 PBoV 에 관한 새로운 정보로 타액을 통한 바이러스 배출 사실을 규명하게 되었다.

주요어: 돼지 bocavirus, 임상증상, 연령별, ORF3

학 번 : 2012-21551