Acta virologica **62**: 343 – 349, 2018 doi:10.4149/av_2018_401

Molecular detection and genetic diversity of porcine bocavirus in piglets in China

Q. MENG[1#], M. QIAO[1#], S. GONG[1#], L. TIAN[1], CH. LI[1], J. QIAO[1*], D. MENG[1], Y. WU[1], K. CAI[2], Z. ZHANG[3], X. CAI[4]

[1]College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, 832003, P. R. China; [2]Urumqi Animal Disease Control and Diagnosis Center, Urumqi, Xinjiang, 830063, P. R. China; [3]Changji Animal Disease Control and Diagnosis Center, Changji, Xinjiang, 831100, P. R. China; [4]State Key Lab of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, 730046, P. R. China

Received October 6, 2017; revised March 12, 2018; accepted September 3, 2018

Summary. – Porcine bocavirus (PBoV) is a recently discovered, non-enveloped and single-stranded DNA virus that can infect pigs. In order to understand PBoV infection and its genetic characterization in piglets in Xinjiang China, PBoV was detected by PCR in 156 clinical samples from 1-month-old piglets. PBoV was detected in 9 clinical samples, with a prevalence rate of 5.77% (9/156). Then nonstructural protein NS1 gene was amplified, sequenced and used for phylogenetic analysis. The results showed that the prevalence rate in the sick piglets was 9.33% (7/75), which is significantly higher than that in the healthy piglets (2.47%, 2/81). The nucleotide sequences of NS1 gene share high identities (96.1–99.2%) within the same groups of PBoVs. Phylogenetic analysis based on complete nucleotide sequence of NS1 gene showed that PBoV strains can be classified into three genetic groups, among which group I contains PBoV1 strains, group II contains PBoV2 strains, and group III contains PBoV3, PBoV4 and PBoV5 strains. Porcine/XJ-12, porcine/XJ-27, porcine/XJ-65, and porcine/XJ-145 had close genetic distance with subgroup 1, belonging to group I; strains porcine/XJ-79 and porcine/XJ-134 were clustered with subgroup 2, belonging to group II, while porcine/XJ-8, porcine/XJ-52 and porcine/XJ-96 were clustered with subgroup 3, which belonged to group III. This study demonstrated for the first time that PBoV strains in Xinjiang belong to three subgroups of three different genetic groups, indicating a substantial genetic diversity of the epidemic strains circulating in China, which provided the useful epidemiological data for scientific control and prevention of this disease in farm pigs.

Keywords: porcine bocavirus; molecular detection; genetic characterization; piglets

Introduction

Bocavirus belongs to the family *Parvoviridae* and the subfamily *Parvovirus* (Fauquet and Fargette, 2005). *Parvovirus* subfamily is divided into six genera: parvovirus, bocavirus, erythroparvovirus, dependoparvovirus, amdoparvovirus and hokovirus (Manteufel and Truyen, 2008). At present, bocavirus includes 5 members: bovine parvovirus (BPV), gorilla bocavirus (GBoV), canine minute virus (CMV), porcine bocavirus (PBoV) and human bocaviruses (HBoV) (Lau *et al.*, 2008). Bocavirus is a non-enveloped, single stranded DNA virus with a genome size of 4786–5905 bp and a diameter of 25–30 nm. Similar to the members of other *Parvovirus* subfamily, PBoV genome has three open reading frames (ORF1~ORF3), which encode non-structural protein (NS1), VP1/VP2 and non-structural protein (NP1) (Yang *et al.*, 2012). Based on the identities of NS1 nucleic acid sequences, PBoV species were proposed to be classified into different clades (PBoV1~PBoV5) (Cságola *et al.*, 2012; Li *et al.*, 2012; Yang *et al.*, 2012; Xiao *et al.*, 2013).

*Corresponding author. E-mail: qj710625@126.com; phone: +86-993-2055036. #These authors contribute equally.

Abbreviations: PBoV = porcine bocavirus; NS1 = nonstructural protein 1; BPV = bovine parvovirus; GBoV = gorilla bocavirus; CMV = canine minute virus; HBoV = human bocaviruses; PMWS = postweaning multisystemic wasting syndrome

In 2009, Swedish researchers for the first time isolated PBoV from lymph nodes of the piglets with postweaning multisystemic wasting syndrome (PMWS) (Blomström *et al.*, 2009). Since then, PBoV was identified in many countries including the USA, Northern Ireland, Cameroon, Uganda, Korea and some European countries (Cadar *et al.*, 2011; McKillen *et al.*, 2011; McNair *et al.*, 2011; Shan *et al.*, 2011b; Blomström *et al.*, 2013; Ndze *et al.*, 2013; Choi *et al.*, 2014; Huang *et al.*, 2014; Jiang *et al.*, 2014; Gunn *et al.*, 2015). In China, PBoV has also been identified in many regions including Hubei, Shanghai, Jiangsu, Shandong, Guizhou and Hong Kong (Cheng *et al.*, 2010; Zhai *et al.*, 2010; Fu *et al.*, 2011; Zhang *et al.*, 2011; Wang *et al.*, 2013; Luo *et al.*, 2015). Xinjiang province is one of the major livestock breeding bases in China. In recent years, with the development of pig industry in Xinjiang, lots of pigs are introduced from abroad. In 2016, the number of slaughtered pigs reached 12.8 million. However, with the increase in swine herds in Xinjiang, a number of new infectious diseases occur frequently. So far, it is still unclear if PBoV infection was prevalent in the piglets in Xinjiang. Furthermore, molecular characteristics of PBoV epidemic strains are also unknown.

The main purpose of this study was to determine the infection rate of PBoVs in the piglets in Xinjiang by PCR method. Genetic characterization of the epidemic strains circulating in Xinjiang China were also performed by phylogenetic analysis, which will provide valuable epidemiological data for the control and prevention of the disease.

Materials and Methods

Collection of clinical samples. From January 2014 to December 2016, a total of 156 clinical samples including 81 samples (49 feces and 32 sera) from healthy piglets (non-diarrhea), and 75 samples (42 feces, 11 sera, 13 lymph nodes, and 9 lungs) from sick piglets (diarrhea). The clinical samples were collected and stored at 4°C in the sterilized 20 ml centrifuge tubes and transported to the Key Laboratory of Animal Disease Prevention and Control of Shihezi University.

Design of specific primers. Based on the nucleotide sequences of NS1 genes in clades PBoV1, PBoV2, PBoV3, PBoV4 and PBoV5 (Table 1), five pairs of diagnostic primers (DF1-DR1, DF2-DR2, DF3-DR3, DF4-DR4 and DF5-DR5) were designed for PCR detection of the relatively conserved region of NS1 gene of each clade of PBoV. Meanwhile, other five pairs of primers (CF1-CR1, CF2-CR2, CF3-CR3, CF4-CR4 and CF5-CR5) were designed to amplify the complete nucleotide sequences of NS1 genes of different clades of PBoV epidemic strains. The sequences of the primers and the expected size of the products by corresponding primers are shown in Table 1.

Sample preparation and DNA extraction. Tissues with appropriate size (lungs, lymph nodes) were placed into 2 ml sterile centrifuge tubes and sterilized PBS (0.01 mol/l, pH 7.2) was added in a volume ratio of 1:5. Tissues were homogenized, and the homogenates were subjected to three freeze and thaw cycles. Following centrifugation for l0 min at 5,000 rpm, the supernatant was collected. Serum samples were collected directly after centrifu-

Table 1. Primers for PCR detection and amplification of complete nucleotide sequences of NS1 genes of PBoVs used in this study

PBoV clades	Primer's	Nucleotide sequence (5' to 3')	Position in reference sequence	Size of amplified product (bp)	Reference sequence (GenBank No.)
PBoV 1	DF1	ATAATAATGATGGAAAGCCAA	1017-1037	600	HQ291308
	DR1	TTAGTTGAACCACTCTGTCTTT	1596-1616		
PBoV 2	DF2	ACACTGGGTGTGCTGCGTGCT	1235-1255	600	HQ291309
	DR2	ATGCAGCGTCCAGTCCTGCGA	1814-1834		
PBoV 3	DF3	AAGCTCAAGAATCATCACGGG	208-228	601	JF713715
	DR3	CGCTGTTCATGACCAGCCGGT	788-808		
PBoV 4	DF4	AGTTGGGCTGTCTGATCAGGA	213-233	603	JF512473
	DR4	AGACAGGTGACGGTCACATCCTGATGAGC	786-815		
PBoV 5	DF5	GACATCGCCGTTCGACGGCTC	301-321	600	JN831651
	DR5	CTCCCCAGCGCGCCATCTTAT	880-900		
PBoV 1	CF1	ATGCCTCTGAACAACTTTCAAGCCGCATTTGAA	489-521	1908	HQ291308
	CR1	TTACTTACGTCCGTCGTCCCCAG	2374-2396		
PBoV 2	CF2	ATGGAGTGCTTCGATCTGGGAGAATACTCTACC	100-132	2111	HQ291309
	CR2	CTAGACGCTCGCTTCGTCTTTTG	2188-2210		
PBoV 3	CF3	ATGAAGCTCAAGAATCATCACGGGCTC	70-96	1754	JF713715
	CR3	TCATGAGATGATCCCATCCCGCC	1801-1823		
PBoV 4	CF4	ATGGCTTCTGCTGGAGTTGGGCTGTC	199-224	2004	JF512473
	CR4	TCATGAGATGATCCCATCCCGC	2181-2202		
PBoV 5	CF5	ATGGCTTCTGCTGGAGTTGGGC	199-221	1981	JN831651
	CR5	TCATGAGATGATCCCATCCCACC	2157-2179		

gation. Stool samples were diluted with sterilized PBS at the ration of 1:3 and centrifuged at 5,000 rpm for 10 min. The supernatant was collected for DNA extraction using MiniBEST viral RNA/DNA extraction kit (TaKaRa, Japan) following the instruction. The extracted DNA was stored at -20°C until use.

PCR detection. PCR was performed using specific primers for each group of the PBoV. PCR reactions (50 μl) containing 2 U of Taq polymerase (TakaRa, Japan), 5 μl of 10×PCR buffer, 4 μl of dNTP (2.5 mmol/l), 1 μl of DNA, and 1 μl DF-DR primers (25 mmol/l) were performed for 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 40 s at 45°C, 30 s at 72°C, and 1 cycle for 10 min at 72°C. PCR products were analyzed by electrophoresis with 1.5% agarose.

Cloning, sequencing and comparing of NS1 genes. After PCR detection, the complete nucleotide sequences of NS1 genes of different PBoV epidemic strains were amplified from PCR positive samples with CF-CR primers. PCR reaction system (50 μl) was the same as mentioned above. PCR reactions were performed for 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 40 s at 50°C, 2 min at 72°C, and 1 cycle for 10 min at 72°C. Then PCR products were analyzed by electrophoresis with 1.0 % agarose. After electrophoresis, PCR products were purified using DNA gel extraction kit (Qiagen, Germany) and ligated into linearized vector pGEM-T easy (Promega, USA), which was subsequently transformed into *Escherichia coli* DH5α. Four colonies were randomly picked for sequencing. Phylogenetic analysis was performed for strains with consistent sequences in at least 3 clones.

Phylogenetic analysis. The complete nucleotide sequences of NS1 genes for PBoVl~PBoV5, BPV, CMV, and HBoV were obtained from GenBank (Supplementary Table 1). The nucleotide sequences of NS1 for PBoV Xinjiang strains were compared with those sequences by Clustal W method using DNAStar software. Neighbor-Joining method (bootstrap repeated 1000 times) was used to calculate genetic distance, and phylogenetic tree was constructed by MEGA 6.0 software (Tamura *et al.*, 2013).

Statistical analysis. Statistical analysis was conducted using SAS software (Version 9.1, SAS Institute, Inc., Cary, NC). A comparison of the PCR positive rate between the healthy and diseased piglets was performed using the χ^2 test. The value of $P < 0.05$ was considered statistically significant.

Results

PCR detection

PBoV was detected by PCR (with expected size, Fig. 1) in 9 samples in 156 samples with an overall positive rate of 5.77% (9/156) (Table 2). Four samples were positive with primer pair DF1-DR1, 2 samples were positive with primer pair DF2-DR2 and DF3-DR3, respectively and 1 sample was positive with primer pair DF4-DR4. The results showed that the PBoV positive rate in the diseased piglets was 9.33% (7/75), which was higher than that (2.47%, 2/81) in the healthy piglets (Table 2).

Sequencing and comparing of the complete NS1 gene sequences of PBoV strains

The complete nucleotide sequences of NS1 genes of different PBoV epidemic strains were successfully amplified from PCR positive samples (four samples were positive with primer pair CF1-CR1, 2 samples were positive with primer pair CF2-CR2 and CF3-CR3, and 1 sample was positive with

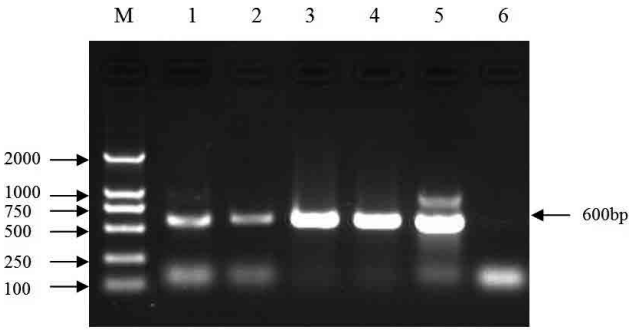

Fig. 1

Detection of PBoV by PCR in partial piglet samples from China
M: DNA marker DL-2000 (2,000, 1,000, 750, 500, 250, 100 bp); 1, 2: PBoV 1 positive sample; 3: PBoV 2 positive sample; 4: PBoV 3 positive sample; 5: PBoV 4 positive sample; 6: Negative sample.

Table 2. Detection results of PBoVs infection by PCR in piglets in China

Clinical status	No. positive / No. tested from the indicated samples (%)				Total positive/total tested (%)
	feces	sera	lymph nodes	lungs	
Clinically healthy (non-diarrhea) (n = 81)	1/49 (2.04)	1/32 (3.13)	0 (0)	0 (0)	2/81 (2.47)
Clinically sick (diarrhea) (n = 75)	3/42 (7.14)	1/11 (9.09)	2/13 (15.38)	1/9 (11.11)	7/75 (9.33)
Total (n = 156)	4/91 (4.40)	2/43 (4.65)	2/13 (15.38)	1/9 (11.11)	9/156 (5.77)

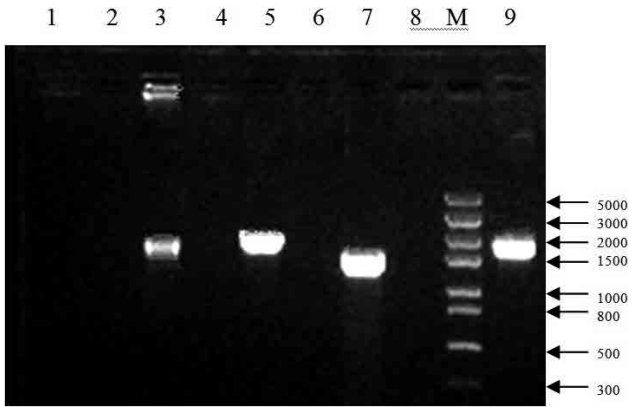

Fig. 2

Amplification of the NS1 gene complete nucleotide sequences of different PBoV epidemic strains from piglet samples
M: Trans 5K DNA marker (5,000, 3,000, 2,000, 1,500, 1,000, 800, 500, 300 bp); 1, 2, 4, 6, 8: Negative samples 3: PBoV 1 positive sample; 5: PBoV 2 positive sample; 7: PBoV 3 positive sample; 9: PBoV 4 positive sample.

primer pair CF4-CR4, respectively) (Fig. 2). The complete nucleotide sequences of NS1 genes of 9 Xinjiang strains (GenBank Acc. Nos.: procine/XJ-12, KU980926; procine/XJ-27, KU980927; procine/XJ-65, KU980929; procine/XJ-145, KU980933; procine/XJ-79, KU980930; procine/XJ-134, KU980932; procine/XJ-8, KU980925; procine/XJ-52, KU980928; procine/XJ-96, KU980931) were obtained and compared with those of 32 representative strains of BoVs (Supplementary Table 1). The results showed that NS1 sequences share high identities (96.1~99.2 %) within the same groups of PBoVs. In contrast, NS1 had low sequence identities between different subgroups of PBoV (37.5~84.8 %). The NS1 shared 36.8~96.2 % nucleotide sequence identities and 32.5~97.6 % amino acid sequence identities among the 9 Xinjiang epidemic strains, indicating a significant genetic diversity of PBoV Xinjiang epidemic strains. Furthermore, NS1 had a nucleotide sequence identity of 27.2~33.1 % with BPV and CMV, while it had identities of 34–48 % with HBoVs (Supplementary Table 2).

Table 3. Genetic characterization of the epidemic strains of PBoVs circulating in China

Strains of PBoV	Lineage	Group	Subgroup
porcine/XJ-12	I	I	1
porcine/XJ-27	I	I	1
porcine/XJ-65	I	I	1
porcine/XJ-145	I	I	1
porcine/XJ-79	I	II	2
porcine/XJ-134	I	II	2
porcine/XJ-8	I	III	3
porcine/XJ-52	I	III	3
porcine/XJ-96	I	III	3

Phylogenetic analysis based on the nucleotide sequences of NS1 genes

Phylogenetic tree was constructed based on the complete nucleotide sequence of NS1 gene. The results showed that bocavirus was divided into two genetic lineages: lineage I (BoVs) and lineage II (porcine parvovirus type 4, PPV4) (Fig. 3). PBoVs, belonging to lineages I, were divided into three genetic groups (group I~group III) and four subgroups (Fig. 3). Xinjiang epidemic strains porcine/XJ-12, porcine/XJ-27, porcine/XJ-65, and porcine/XJ-145 had close genetic distance with subgroup 1, belonging to group I; strains porcine/XJ-79, porcine/XJ-134 were clustered with subgroup 2, belonging to group II, while porcine/XJ-8, porcine/XJ-52 and porcine/XJ-96 were clustered with subgroup 3, which belonged to group III (Table 3). Our results demonstrated that Xinjiang epidemic strains of PBoV belong to 3 different subgroups in the three groups, which exhibit substantial genetic diversity.

Discussion

So far, there is no internationally recognized genotyping criterion for PBoVs. Based on the homology of NS1 gene of PBoV, some studies classified different PBoV epidemic strains into different clades (PBoV1~PBoV5) (Xiao *et al.*, 2013; Choi *et al.*, 2014). PBoV1 with a genome size of 4,786 bp was detected in pigs suffering from PMWS in Sweden (Blomström *et al.*, 2009), which suggested that PoBoV1 might play some role as co-factor in the development of the disease. PBoV2 has a genome size of 5,780–5,905 bp and is present in pigs in China (Cheng *et al.*, 2010; Shan *et al.*, 2011a), while PBoV3, PBoV4 and PBoV5 have a genome sizes of 5,173–5,186 bp, 4,152bp and 5,076 bp, respectively (Li *et al.*, 2012). Our results showed that PBoV epidemic strains can be classified into three genetic groups among which group I contains PBoV1 clade, group II contains PBoV 2 clade, and group III contains PBoV3, PBoV4 and PBoV5 clade. Based on complete nucleotide sequence of NS1 gene, the results showed that strains within the same groups were conserved, while strains in different groups exhibited substantial genetic diversity. The PBoV strains from Xinjiang belong to three different genetic groups and three subgroups, suggesting that the PBoV strains circulating in Xinjiang are also genetically diversified. To our knowledge, this is the first report on genetic diversity of PBoV epidemic strains in Xinjiang China. Further studies will be required to determine

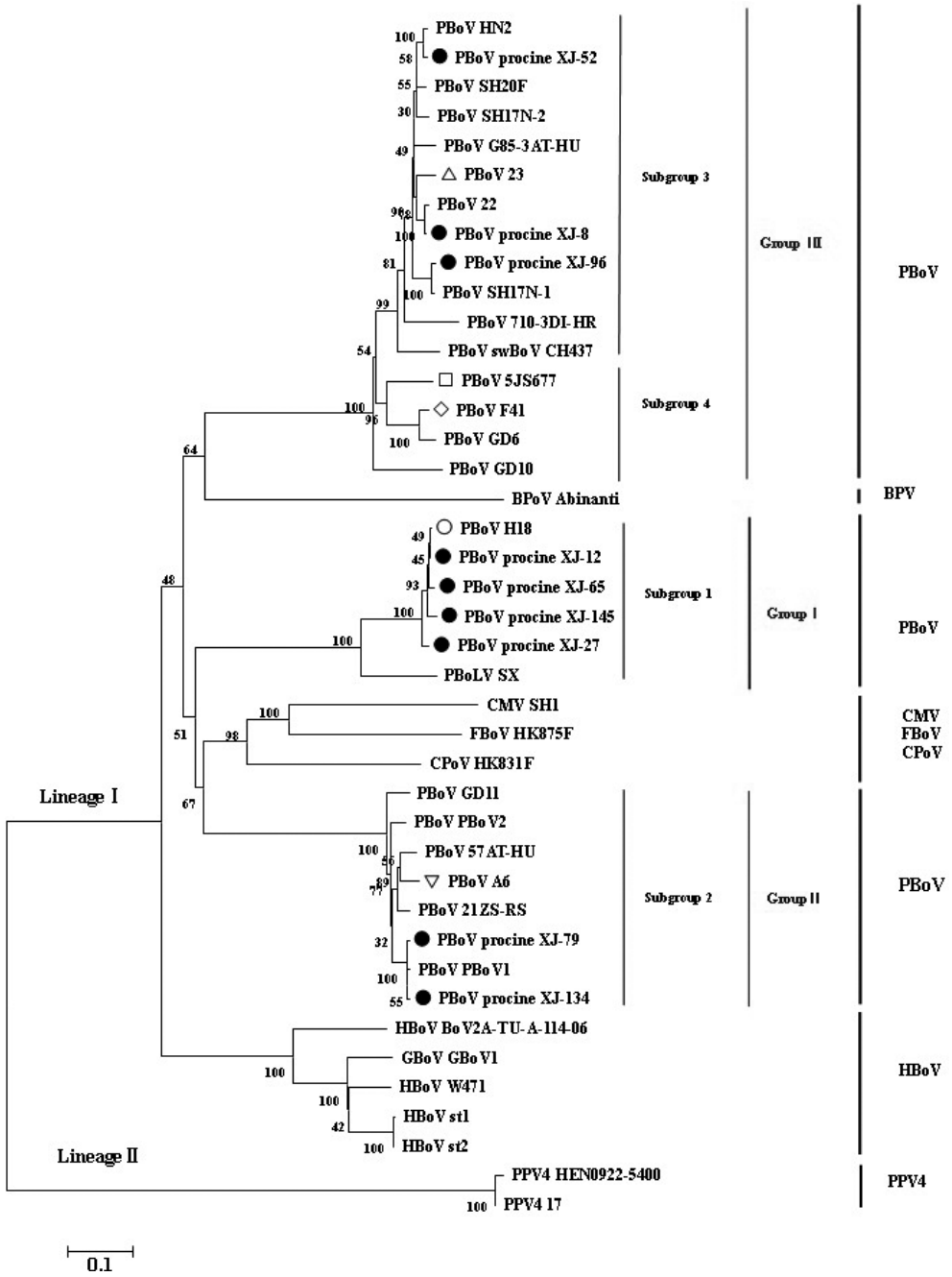

Fig. 3

Phylogenetic analysis of bocavirus strains based on the complete nucleotide sequences of NS1 genes

The nucleotide sequences of NS1 gene obtained in this study and available in GenBank were used to construct phylogenetic tree by the neighbor-joining and maximum-likelihood methods using 1000 bootstrap replicate values. Vertical lines are used to indicate lineages (or groups) which were referred to in the text. The filled circles represent strains of PBoVs identified in this study. The circle, upright triangle, inverted triangle, box and diamond indicate the representative strains of PBoV H18, 23, A6, 5JS677 and F41, respectively. The GenBank Acc. Nos. of different strains of bocavirus in this study were as follows: PBoV Bocavirus pig/SX/China/2010, HQ223038; H18, HQ291308; PBoV2, HM053694; 22, JF713714; F41, JF512473; 5/JS677, JN831651; HEN0922-5400, GU978964; SH17N-1, JF429835; SH17N-2, JF429835; GD11, KM402139; GD10, KM402138; GD6, KM402137; SWBoV CH437, NC_023673;G85-3AT-HU, KF206168; G85-3AT-HU, KF206167; 710-3DI-HR, KF206166; 710-1DI-HR, KF206165; 57AT-HU, KF206160; 5ZS-RS, KF206158; 21ZS-RS, KF206155; 5/JS677, NC_016647; PBoV1, HM053693; HN2 KC473563, SH20F NC_016031; 23, JF713715; A6, HQ291309; SX, HQ223038; procine/XJ-12, KU980926; procine/XJ-27, KU980927; procine/XJ-65, KU980929; procine/XJ-145, KU980933; procine/XJ-79, KU980930; procine/XJ-134, KU980932; procine/XJ-8, KU980925; procine/XJ-52, KU980928; procine/XJ-96, KU980931; BPV Abinanti, DQ335247; CBoV HK831F, JQ692591; HBoV st1, DQ000495; st2, DQ000496; BoV2A-TU-A-114-06, FJ973558; W471, EU918736; HBoV4-NI-385, FJ973561; FBoV HK875F, JQ692587; GBoV GBoV1, HM145750; CMV SH1, FJ899734; KU980925.

whether the pathogenicity and antigenicity of the epidemic strains belonging to different genetic lineages are different.

Previous molecular epidemiological studies showed that pigs had different PBoV infection rate and different geographical distribution of the PBoV subgroups (Zhai *et al*., 2010; Jiang *et al*., 2014; Zhang *et al*., 2015a). Our studies showed that PBoV infection was also present in the piglets in Xinjiang China with an overall infection rate of 5.77%. The subgroup 1 infection rate was relatively higher than other subgroups. In addition, piglets also had co-infections with both PBoV and PPV1 (porcine parvovirus 1) or PCV-2 (porcine circovirus 2) (data not shown). Due to the wide presence of PBoV infection abroad, it is recommended that pigs from epidemic areas of PBoV infection abroad should be subjected to virus detection before being introduced to Xinjiang China.

PBoV is a recently discovered parvovirus, but its biological characteristics are not completely identical to the traditional parvovirus family. Currently, PBoV transmission, epidemiology and pathogenicity are unclear (Li *et al*., 2012; Zhang *et al*., 2015b). Zhai *et al*. found that PBoV infection rate was relatively high (69.7%) in the weaning piglets with clinical respiratory symptoms (Zhai *et al*., 2010). In contrast, PBoV infection rate in other piglets was relatively low (13.6%), suggesting that PBoV infection may be correlated with respiratory diseases. We showed here that PBoV infection rate in the feces of piglets (2.47%) with diarrhea was significantly higher than that in the healthy piglets (9.33%), suggesting that PBoV infection may also be correlated with gastrointestinal diseases. However, molecular mechanisms of PBoV infection and immunity are still unclear (Zhou *et al*., 2014; Zhang *et al*., 2015b, 2016). Further studies (e.g., virus isolation and experimental infection) are needed to reveal whether PBoV is a primary pathogen causing respiratory and intestinal disease or it plays synergistic roles in the infections caused by other pathogens.

In general, in this study it was firstly demonstrated that PBoV strains in Xinjiang belong to three subgroups of three different genetic groups (subgroup 1 of group I, subgroup 2 of group II, and subgroup 3 of group III), indicating a substantial genetic diversity of the epidemic strains circulating in China, which provided the useful epidemiological data for scientific control and prevention of this disease in farmed pigs.

Acknowledgments. The authors thank the field staff for providing the samples for this study. This work was supported by grant from the national key research and development program (No. 2017YFD0502300), International Science & Technology Cooperation Program of China (No. 2014DFR31310); Grant from Youth Science and Technology Innovation Leader of Xinjiang Production and Construction Corps (No. 2016BC001).

Supplementary informations is available in the online version of the paper.

References

Blomström AL, Belák 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 Res. 146, 125–129. https://doi.org/10.1016/j.virusres.2009.09.006

Blomström AL, Ståhl K, Okurut AR, Masembe C, Berg M (2013): Genetic characterisation of a porcine bocavirus detected in domestic pigs in Uganda. Virus Genes 47, 370–373. https://doi.org/10.1007/s11262-012-0855-1

Cadar D, Cságola A, Lorincz M, Tombácz K, Kiss T, Spînu 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. https://doi.org/10.1007/s11262-011-0650-4

Cheng WX, Li JS, Huang CP, Yao DP, Liu N, Cui SX, Jin Y, Duan ZJ (2010): Identification and nearly full-length genome characterization of novel porcine bocaviruses. PLoS One 5, e13583. https://doi.org/10.1371/journal.pone.0013583

Choi MG, Park SJ, Nguyen VG, Chung HC, Kim AR, Park BK (2014): Molecular detection and genetic analysis of porcine bocavirus in Korean domestic swine herds. Arch. Virol. 159, 1487–1492. https://doi.org/10.1007/s00705-013-1944-8

Cságola A, Lőrincz M, Cadar D, Tombácz K, Biksi I, Tuboly T (2012): Detection, prevalence and analysis of emerging porcine parvovirus infections. Arch. Virol. 157, 1003–1010. https://doi.org/10.1007/s00705-012-1257-3

Fauquet CM, Fargette D (2005): International Committee on Taxonomy of Viruses and the 3,142 unassigned species. Virol. J. 2, 64. https://doi.org/10.1186/1743-422X-2-64

Fu X, Wang X, Ni B, Shen H, Wang H, Zhang X, Chen S, Shao S, Zhang W (2011): Recombination analysis based on the complete genome of bocavirus. Virol. J. 8, 182. https://doi.org/10.1186/1743-422X-8-182

Gunn L, Collins PJ, Fanning S, McKillen J, Morgan J, Staines A, O'Shea H (2015): Detection and characterisation of novel bocavirus (genus Bocaparvovirus) and gastroenteritis viruses from asymptomatic pigs in Ireland. Infect. Ecol. Epidemiol. 5, 27270. https://doi.org/10.3402/iee.v5.27270

Huang J, Mor SK, Erber J, Voss E, Goyal SM (2014): Detection and characterization of porcine bocavirus in the United States. Arch. Virol. 159, 1797–1801. https://doi.org/10.1007/s00705-013-1972-4

Jiang YH, Xiao CT, Yin SH, Gerber PF, Halbur PG, Opriessnig T (2014): High prevalence and genetic diversity of porcine bocaviruses in pigs in the USA, and identification of multiple novel porcine bocaviruses. J. Gen. Virol. 95, 453–465. https://doi.org/10.1099/vir.0.057042-0

Lau SK, Woo PC, Tse H, Fu CT, Au WK, Chen XC, Tsoi HW, Tsang TH, Chan JS, Tsang DN, Li KS, Tse CW, Ng TK, Tsang OT, Zheng BJ, Tam S, Chan KH, Zhou B, Yuen KY (2008): Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. J. Gen. Virol. 89, 1840–1848. https://doi.org/10.1099/vir.0.2008/000380-0

Li B, Ma J, Xiao S, Fang L, Zeng S, Wen L, Zhang X, Ni Y, Guo R, Yu Z, Zhou J, Mao A, Lv L, Wang X, He K (2012): Complete genome sequence of a novel species of porcine bocavirus, PBoV5. J. Virol. 86, 1286–1287. https://doi.org/10.1128/JVI.06589-11

Luo Y, Liang L, Zhou L, Zhao K, Cui S (2015): Concurrent infections of pseudorabies virus and porcine bocavirus in China detected by duplex nanoPCR. J. Virol. Methods 219, 46–50. https://doi.org/10.1016/j.jviromet.2015.03.016

Manteufel J, Truyen U (2008): Animal bocaviruses: a brief review. Intervirology 51, 328–234. https://doi.org/10.1159/000173734

McKillen J, McNeilly F, Duffy C, McMenamy M, McNair I, Hjertner B, Millar A, McKay K, Lagan P, Adair B, Allan G (2011): Isolation in cell cultures and initial characterisation of two novel bocavirus species from swine in Northern Ireland. Vet. Microbiol. 152, 39–45. https://doi.org/10.1016/j.vetmic.2011.04.013

McNair I, McNeilly F, Duffy C, McKillen J, McMenamy M, Welsh M, Allan G (2011): Production, characterisation and applications of monoclonal antibodies to two novel porcine bocaviruses from swine in Northern Ireland. Arch Virol. 156, 2157–2162. https://doi.org/10.1007/s00705-011-1107-8

Ndze VN, Cadar D, Cságola A, Kisfali P, Kovács E, Farkas S, Ngu AF, Esona MD, Dán Á, Tuboly T, Bányai K (2013): Detection of novel porcine bocaviruses in fecal samples of asymptomatic pigs in Cameroon. Infect. Genet. Evol. 17, 277–282. https://doi.org/10.1016/j.meegid.2013.03.006

Shan T, Lan D, Li L, Wang C, Cui L, Zhang W, Hua X, Zhu C, Zhao W, Delwart E (2011a): Genomic characterization and high prevalence of bocaviruses in swine. PLoS One 6, e17292. https://doi.org/10.1371/journal.pone.0017292

Shan T, Li L, Simmonds P, Wang C, Moeser A, Delwart E (2011b): The fecal virome of pigs on a high-density farm. J. Virol. 85, 11697–11708. https://doi.org/10.1128/JVI.05217-11

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013): MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. https://doi.org/10.1093/molbev/mst197

Wang E, Liu W, Yang B, Liu J, Ma X, Lan X (2014): Complete sequence and phylogenetic analysis of a porcine bocavirus strain swBoV CH437. Virus Genes 48, 387–390. https://doi.org/10.1007/s11262-013-1032-x

Xiao CT, Halbur PG, Opriessnig T (2013): Molecular evolutionary genetic analysis of emerging parvoviruses identified in pigs. Infect. Genet. Evol. 16, 369–376. https://doi.org/10.1016/j.meegid.2013.03.017

Yang WZ, Yu JM, Li JS, Cheng WX, Huang CP, Duan ZJ (2012): Genome characterization of a novel porcine bocavirus. Arch. Virol. 157, 2125–2132. https://doi.org/10.1007/s00705-012-1407-7

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. Arch. Virol. 155, 1313–1317. https://doi.org/10.1007/s00705-010-0698-9

Zhang HB, Huang L, Liu YJ, Lin T, Sun CQ, Deng Y, Wei ZZ, Cheung AK, Long JX, Yuan SS (2011): Porcine bocaviruses: genetic analysis and prevalence in Chinese swine population. Epidemiol. Infect. 139, 1581–1586. https://doi.org/10.1017/S0950268811000847

Zhang Q, Zhang C, Gao M, He X, Diao Y, Goyal SM, Mor SK, Huang J (2015a): Evolutionary, epidemiological, demographical, and geographical dissection of porcine bocavirus in China and America. Virus Res. 195, 13–24. https://doi.org/10.1016/j.virusres.2014.09.012

Zhang R, Fang L, Wang D, Cai K, Zhang H, Xie L, Li Y, Chen H, Xiao S (2015b): Porcine bocavirus NP1 negatively regulates interferon signaling pathway by targeting the DNA-binding domain of IRF9. Virology 485, 414–421. https://doi.org/10.1016/j.virol.2015.08.005

Zhang R, Fang L, Cai K, Zeng S, Wu W, An K, Chen H, Xiao S (2016): Differential contributions of porcine bocavirus NP1 protein N- and C-terminal regions to its nuclear localization and immune regulation. J. Gen. Virol. 97, 1178–1188. https://doi.org/10.1099/jgv.0.000413

Zhou F, Sun H, Wang Y (2014): Porcine bocavirus: achievements in the past five years. Viruses 6, 4946–4960. https://doi.org/10.3390/v6124946

Acta virologica **62**: 343 – 349, 2018 doi:10.4149/av_2018_401

Supplementary information

Molecular detection and genetic diversity of porcine bocavirus in piglets in China

Q. MENG[1#], M. QIAO[1#], S. GONG[1#], L. TIAN[1], CH. LI[1], J. QIAO[1*], D. MENG[1], Y. WU[1], K. CAI[2], Z. ZHANG[3], X. CAI[4]

[1]College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, 832003, P. R. China; [2]Urumqi Animal Disease Control and Diagnosis Center, Urumqi, Xinjiang, 830063, P. R. China; [3]Changji Animal Disease Control and Diagnosis Center, Changji, Xinjiang, 831100, P. R. China; [4]State Key Lab of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, 730046, P. R. China

Received October 6, 2017; revised March 12, 2018; accepted September 3, 2018

Supplementary Table 1. Information of nucleotide sequences of bocaviruses in this study

Virus	Host	Strain / Isolate	GenBank Acc. Nos.	Country
Porcine bocavirus	Sus scrofa	Bocavirus pig/SX/China/2010	HQ223038	Hubei/China
Porcine bocavirus	Sus scrofa	H18	HQ291308	Shanghai/China
Porcine bocavirus	Sus scrofa	PBoV2	HM053694	Beijing/China
Porcine bocavirus	Sus scrofa	22	JF713714	USA
Porcine bocavirus	Sus scrofa	F41	JF512473	United Kingdom
Porcine bocavirus	Sus scrofa	5/JS677	JN831651	Jiangsu/China
Porcine bocavirus	Sus scrofa	HEN0922-5400	GU978964	Shanghai/China
Porcine bocavirus	Sus scrofa	SH17N-1	JF429835	Hong Kong/China
Porcine bocavirus	Sus scrofa	SH17N-2	JF429835	Hong Kong/China
Porcine bocavirus	Sus scrofa	GD11	KM402139	China
Porcine bocavirus	Sus scrofa	GD10	KM402138	China
Porcine bocavirus	Sus scrofa	GD6	KM402137	China
Porcine bocavirus	Sus scrofa	SWBoV CH437	NC_023673	China
Porcine bocavirus	Sus scrofa	G85-3AT-HU	KF206168	Hungary
Porcine bocavirus	Sus scrofa	G85-3AT-HU	KF206167	Hungary
Porcine bocavirus	Sus scrofa	710-3DI-HR	KF206166	Hungary
Porcine bocavirus	Sus scrofa	710-1DI-HR	KF206165	Hungary
Porcine bocavirus	Sus scrofa	57AT-HU	KF206160	Hungary
Porcine bocavirus	Sus scrofa	5ZS-RS	KF206158	Hungary
Porcine bocavirus	Sus scrofa	21ZS-RS	KF206155	Hungary
Porcine bocavirus	Sus scrofa	5/JS677	NC_016647	Jiangsu/China
Porcine bocavirus	Sus scrofa	PBoV1	HM053693	Beijing/China
Porcine bocavirus	Sus scrofa	HN2	KC473563	Henan/China
Porcine bocavirus	Sus scrofa	SH20F	NC_016031	Hong Kong/China
Porcine bocavirus	Sus scrofa	23	JF713715	USA
Porcine bocavirus	Sus scrofa	A6	HQ291309	Shanghai/China

Virus	Host	Strain / Isolate	GenBank Acc. Nos.	Country
Porcine bocavirus	Sus scrofa	SX	HQ223038	Hubei/China
Porcine bocavirus	Sus scrofa	porcine/XJ-8	KU980925	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-12	KU980926	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-27	KU980927	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-52	KU980928	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-65	KU980929	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-79	KU980930	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-96	KU980931	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-134	KU980932	Xinjiang/China
Porcine bocavirus	Sus scrofa	porcine /XJ-145	KU980933	Xinjiang/China
Bovine parvovirus	Bos taurus	Abinanti	DQ335247	USA
Canine bocaviruses	Canis lupus	HK831F	JQ692591	Hong Kong/China
Human bocaviruses	Homo sapiens	st1	DQ000495	Sweden
Human bocaviruses	Homo sapiens	st2	DQ000496	Sweden
Human bocaviruses	Homo sapiens	BoV2A-TU-A-114-06	FJ973558	USA
Human bocaviruses	Homo sapiens	W471	EU918736	Australia
Feline bocavirus	Felis catus	HK875F	JQ692587	Hong Kong/China
Gorilla bocavirus	Gorilla gorilla	GBoV1	HM145750	USA
Canine minute virus	Canine	SH1	FJ899734	China

Supplementary Table 2. Identities of nucleotide sequences of NS1 gene among different strains of bocaviruses in this study

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	
1		18.5	23.0	20.8	17.3	22.2	21.7	21.7	21.9	21.3	25.9	24.2	20.4	21.3	22.2	19.6	21.5	18.1	22.3	20.4	20.8	21.4	23.4	19.2	24.2	21.4	21.5	21.5	21.5	26.8	21.4	21.5	34.4	23.4	23.5	23.5	23.5	31.0	22.2	22.2	22.2	1
2	112.0		59.5	49.4	23.8	24.8	29.9	29.9	51.3	47.7	24.4	24.9	27.1	22.0	22.5	26.4	22.6	19.4	15.0	43.1	22.4	42.0	22.0	42.6	43.2	42.0	41.8	41.8	42.1	25.1	42.1	41.8	24.6	19.4	19.5	22.3	22.1	21.9	46.5	46.5	46.5	2
3	103.2	63.1		46.9	23.3	24.5	30.3	30.3	29.1	43.6	50.2	52.4	39.3	29.8	28.7	44.9	24.1	37.2	29.9	39.5	23.5	39.4	45.2	39.6	39.8	39.5	39.6	39.6	39.4	51.1	29.5	39.6	49.8	29.2	29.1	45.0	27.0	23.8	30.4	30.4	30.4	3
4	104.2	61.6	69.3		24.8	24.5	29.7	29.7	30.3	40.8	23.3	26.2	37.3	22.8	21.0	37.0	22.6	19.1	20.5	36.9	22.8	48.5	20.7	36.5	36.5	48.0	48.5	48.5	44.0	23.6	43.8	48.5	23.4	20.8	20.9	21.1	20.8	22.6	34.1	34.1	34.1	4
5	105.4	94.3	98.6	98.3		73.2	86.6	86.5	85.2	27.0	40.7	41.5	24.0	36.9	29.9	24.4	37.1	30.4	21.4	25.2	37.1	30.9	36.0	24.3	24.3	30.9	31.4	31.4	31.0	41.3	30.9	31.4	40.8	36.5	36.6	36.8	36.3	36.3	41.2	41.2	41.2	5
6	102.6	94.0	97.2	102.0	34.3		74.4	74.4	73.2	28.7	50.4	53.7	25.1	32.8	30.4	21.4	40.1	39.2	21.0	20.2	46.1	32.4	46.7	26.0	25.9	32.3	32.1	32.1	32.3	51.1	32.4	32.1	52.9	47.3	47.2	45.5	46.8	46.9	27.8	27.8	27.8	6
7	93.4	81.7	82.7	86.0	14.7	32.3		99.7	87.4	27.2	48.9	41.9	26.9	37.9	31.3	27.0	38.4	37.4	22.1	28.1	38.6	31.1	39.1	26.6	27.1	29.8	31.4	31.4	31.1	49.8	31.1	31.4	40.2	38.2	38.3	38.1	45.3	38.4	29.6	29.6	29.6	7
8	93.6	82.2	83.1	86.2	14.8	32.3	0.3		87.2	27.2	49.2	41.8	26.9	37.9	31.3	27.0	38.4	37.4	22.1	28.1	38.6	31.2	38.9	26.6	27.1	29.8	31.6	31.6	31.2	49.9	31.2	31.6	40.2	38.1	38.2	38.0	45.5	38.4	29.6	29.6	29.6	8
9	92.6	82.8	86.8	87.6	16.0	32.7	14.0	14.2		27.6	41.8	42.2	25.8	37.8	26.2	26.9	37.4	37.8	22.2	26.6	37.5	30.8	37.6	26.9	27.0	30.8	31.2	31.2	30.8	42.5	30.8	31.2	41.6	38.1	38.2	38.1	37.6	37.8	30.7	30.7	30.7	9
10	94.0	89.9	73.8	86.0	95.6	96.5	86.2	86.6	91.5		51.0	52.3	92.5	33.8	36.7	93.0	28.0	47.8	51.9	92.3	27.7	41.6	49.2	92.2	92.6	41.7	41.2	41.2	41.5	53.5	41.8	41.2	50.9	48.1	47.6	47.5	48.0	36.6	41.2	41.2	41.2	10
11	82.6	93.8	77.2	86.4	79.2	74.4	75.8	76.0	76.3	68.8		95.2	50.0	81.7	88.5	50.3	83.0	94.3	81.7	51.0	82.4	25.1	96.1	51.1	51.5	25.1	24.6	24.6	25.3	96.0	25.1	24.6	99.0	94.6	95.2	96.1	95.8	89.2	24.4	24.4	24.4	11
12	81.9	89.5	76.4	83.8	78.2	72.7	75.1	75.3	76.0	68.3	5.0		52.2	83.8	88.1	52.3	84.4	93.2	82.7	52.3	83.5	25.6	94.0	52.8	52.8	25.6	25.2	25.2	25.7	94.6	25.6	25.2	95.7	92.6	93.0	94.3	94.6	88.9	25.1	25.1	25.1	12
13	95.3	97.5	78.6	92.7	100.1	97.6	89.5	89.9	93.6	6.0	71.8	69.5		32.3	34.2	94.4	26.7	43.6	49.1	92.7	26.6	41.1	45.7	92.7	93.5	41.1	40.8	40.8	41.0	51.9	41.2	40.8	50.6	46.0	45.6	45.1	45.9	26.1	40.1	40.1	40.1	13
14	82.9	94.4	86.1	89.2	81.8	78.0	78.8	78.9	80.9	73.6	21.0	18.6	75.5		81.3	33.7	86.7	80.6	79.8	46.4	85.6	27.5	81.2	48.4	34.8	27.5	27.0	27.0	27.6	81.9	27.5	27.0	82.1	81.9	81.4	81.3	81.5	80.7	26.9	26.9	26.9	14
15	86.7	98.3	84.1	92.1	82.5	80.3	81.5	81.7	81.6	73.9	13.3	13.0	77.3	21.6		33.8	82.3	88.3	81.0	34.7	81.6	27.6	89.2	34.9	35.0	27.6	27.3	27.3	27.8	90.2	27.6	27.3	88.9	87.7	88.0	88.7	89.0	84.3	23.0	23.0	23.0	15
16	93.8	95.7	75.3	89.3	96.7	97.0	90.8	91.2	93.8	5.2	72.6	70.0	6.0	77.8	78.0		26.3	43.2	48.7	93.0	26.1	41.6	45.0	93.9	94.2	41.5	41.3	41.3	41.6	51.4	41.6	41.3	50.1	45.9	45.6	44.7	45.1	35.1	40.7	40.7	40.7	16
17	83.2	96.4	84.8	91.1	78.8	74.9	78.7	78.9	79.4	72.4	19.4	17.6	75.4	15.0	20.2	76.6		81.6	81.1	26.8	96.2	27.7	82.2	46.7	26.8	27.7	27.2	27.2	27.9	82.9	27.7	27.2	83.1	83.1	82.4	82.1	82.1	82.2	25.2	25.2	25.2	17
18	100.6	105.8	92.2	103.2	92.7	89.1	77.7	77.8	80.6	75.6	5.9	7.1	78.6	22.7	13.1	79.7	21.5		80.8	43.6	80.5	25.5	94.4	43.8	43.8	25.5	23.8	23.8	25.6	94.9	25.5	23.8	94.8	92.4	92.6	95.3	95.6	87.7	23.4	23.4	23.4	18
19	85.5	100.1	82.4	100.3	85.0	81.5	84.0	84.1	82.4	65.3	21.1	19.5	70.0	23.6	21.9	72.1	21.8	21.7		46.9	80.8	31.0	81.6	50.0	50.4	28.2	30.8	30.8	31.0	82.3	31.0	30.8	82.1	81.6	81.6	81.1	81.9	80.0	22.7	22.7	22.7	19
20	92.9	92.3	76.8	89.8	94.7	96.3	88.4	88.8	91.8	6.3	70.4	68.9	7.8	74.8	76.4	7.3	74.5	76.8	70.1		26.6	42.5	45.9	92.7	94.6	42.4	42.2	42.2	42.5	52.4	42.6	42.2	51.3	45.9	46.0	45.3	45.9	45.0	41.5	41.5	41.5	20
21	84.0	94.8	86.0	89.9	78.5	74.9	78.6	78.7	79.3	73.4	20.2	18.8	75.7	16.1	21.0	77.1	4.0	22.7	22.3	75.7		27.6	81.0	45.9	26.7	27.6	27.1	27.1	49.5	81.6	27.6	27.1	82.6	82.3	81.7	81.0	81.0	81.4	25.3	25.3	25.3	21
22	96.5	71.6	80.9	72.6	84.5	85.9	86.1	86.2	86.4	74.4	80.1	79.1	80.2	82.1	84.4	78.4	81.7	82.1	86.7	76.6	80.6		23.2	41.0	41.0	99.3	98.8	98.8	98.8	25.2	98.6	98.8	25.0	23.1	23.2	24.6	23.4	23.3	77.7	77.7	77.7	22
23	84.6	98.0	79.8	93.5	81.2	76.7	77.1	77.3	78.6	68.3	4.0	6.3	74.8	22.0	12.5	75.6	20.6	5.8	20.9	73.7	22.2	87.0		46.1	46.2	23.0	22.7	22.7	23.1	99.0	23.0	22.7	96.6	94.2	94.7	96.1	96.3	88.8	26.9	26.9	26.9	23
24	94.2	94.9	77.3	92.7	98.7	97.3	92.7	93.1	94.6	6.2	69.5	68.4	8.0	76.4	76.1	6.5	75.9	77.6	70.6	7.8	76.5	81.0	72.9		94.3	41.4	41.0	41.0	41.1	53.7	41.2	41.0	51.4	46.2	45.7	46.0	46.4	44.8	40.9	40.9	40.9	24
25	92.8	94.7	76.6	89.5	96.0	96.1	87.2	87.6	92.2	5.7	69.0	67.6	6.8	74.5	76.4	6.0	74.7	77.2	69.8	5.7	75.2	80.9	73.5	6.1		41.1	40.9	40.9	41.3	53.3	41.2	40.9	51.5	46.7	46.2	46.3	46.3	44.9	40.9	40.9	40.9	25
26	96.0	71.4	81.0	73.2	84.4	85.8	86.2	86.4	86.2	74.0	79.7	78.9	79.8	81.8	84.0	78.1	81.4	81.8	86.6	76.3	80.4	0.7	86.6	80.4	80.4		98.1	98.1	98.1	25.2	97.9	98.1	25.0	23.1	23.2	31.5	23.4	23.3	78.4	78.4	78.4	26
27	96.5	72.6	80.2	72.8	82.9	86.8	85.0	85.2	85.1	75.6	81.9	80.5	81.2	83.7	85.5	79.2	83.5	83.2	87.8	77.3	82.2	1.2	88.3	81.7	81.6	2.0		100.0	97.6	25.0	97.4	100.0	24.6	23.0	23.0	27.7	23.0	24.5	78.9	78.9	78.9	27
28	96.5	72.6	80.2	72.8	82.9	86.8	85.0	85.2	85.1	75.6	81.9	80.5	81.2	83.7	85.5	79.2	83.5	83.2	87.8	77.3	82.2	1.2	88.3	81.7	81.6	2.0	0.0		97.6	25.0	97.4	100.0	24.6	23.0	23.0	27.7	23.0	24.5	78.9	78.9	78.9	28
29	96.2	72.0	81.2	72.4	85.0	86.6	86.8	87.0	86.9	75.5	80.7	79.7	80.9	83.1	84.1	78.9	81.9	82.8	87.5	76.6	80.1	1.3	88.3	81.7	80.7	2.0	2.5	2.5		25.2	97.4	97.6	25.2	23.2	23.3	24.7	23.5	23.3	78.9	78.9	78.9	29
30	81.1	91.9	75.2	86.9	79.5	74.3	75.2	75.4	75.5	67.6	4.1	5.6	70.0	21.0	10.9	70.8	19.5	5.3	20.2	68.3	21.3	82.0	1.0	68.1	67.7	81.6	83.1	83.1	83.5		25.0	24.8	96.7	94.1	94.4	96.4	97.1	89.5	29.5	29.5	29.5	30
31	97.4	71.2	80.5	72.7	84.6	86.0	86.6	86.8	86.6	74.1	80.5	79.5	79.8	82.3	83.8	77.6	82.1	82.4	86.8	76.6	80.9	1.4	87.2	80.6	80.5	2.2	2.7	2.7	2.7	82.5		97.4	25.0	23.1	23.2	24.6	23.4	23.3	79.0	79.0	79.0	31
32	96.5	72.6	80.2	72.8	82.9	86.8	85.0	85.2	85.1	75.6	81.9	80.5	81.2	83.7	85.5	79.2	83.5	83.2	87.8	77.3	82.2	1.2	88.3	81.7	81.6	2.0	0.0	0.0	2.5	83.1	2.7		24.6	23.0	23.0	27.7	23.0	24.5	78.9	78.9	78.9	32
33	82.0	92.7	77.4	86.2	79.6	73.9	75.5	75.7	76.3	68.5	1.0	4.5	71.2	20.7	12.5	72.1	19.3	5.4	20.2	70.1	20.0	80.5	3.5	69.6	68.6	80.3	82.1	82.1	81.2	3.4	81.0	82.1		94.3	94.9	95.9	96.3	89.4	24.6	24.6	24.6	33
34	83.8	98.1	81.6	91.4	81.4	76.7	79.0	79.2	79.8	71.4	5.7	7.8	77.2	21.0	13.9	76.7	19.2	8.0	21.1	75.6	20.3	84.2	6.0	75.0	74.5	83.9	84.7	84.7	85.2	6.1	84.7	84.7	6.0		98.8	94.1	94.2	87.3	27.1	27.1	27.1	34
35	84.0	97.9	81.8	90.9	81.3	76.6	78.3	78.5	79.4	70.9	5.0	7.4	76.8	21.7	13.5	76.0	20.1	7.8	21.2	75.1	21.1	84.1	5.6	74.8	74.2	83.7	84.9	84.9	85.0	5.9	84.6	84.9	5.3	1.3		94.0	94.2	87.6	27.1	27.1	27.1	35
36	84.8	94.8	81.9	90.6	79.5	74.5	77.5	77.7	78.1	70.2	4.0	5.9	77.3	21.8	12.7	77.4	20.5	4.9	21.6	75.6	22.1	84.7	4.0	74.9	74.7	84.3	85.2	85.2	85.8	3.7	84.9	85.2	4.1	6.2	6.3		97.0	89.0	27.6	27.6	27.6	36
37	83.7	96.0	81.5	92.2	82.3	76.6	78.5	78.7	79.5	68.6	4.2	5.6	74.2	21.5	12.4	74.8	20.7	4.6	20.5	73.3	22.2	85.1	3.8	72.9	72.6	84.7	86.1	86.1	86.3	3.0	85.4	86.1	3.7	6.0	6.0	3.1		89.5	27.1	27.1	27.1	37
38	83.8	95.2	82.4	88.6	80.2	76.3	78.3	78.0	77.8	71.8	11.8	12.1	76.6	22.7	18.0	77.1	20.4	13.5	23.2	76.6	21.5	86.7	12.3	77.1	76.4	86.7	88.1	88.1	87.6	11.4	87.4	88.1	11.4	14.2	13.8	12.1	11.4		26.8	26.8	26.8	38
39	95.6	74.4	77.6	76.9	83.4	87.8	83.8	84.0	82.9	74.7	84.1	82.3	81.4	84.2	83.8	79.8	85.7	85.1	90.5	78.0	84.5	27.4	88.0	81.8	80.9	26.4	25.7	25.7	25.6	82.4	25.4	25.7	83.8	86.9	86.7	86.1	88.0	90.9		100.0	100.0	39
40	95.6	74.4	77.6	76.9	83.4	87.8	83.8	84.0	82.9	74.7	84.1	82.3	81.4	84.2	83.8	79.8	85.7	85.1	90.5	78.0	84.5	27.4	88.0	81.8	80.9	26.4	25.7	25.7	25.6	82.4	25.4	25.7	83.8	86.9	86.7	86.1	88.0	90.9	0.0		100.0	40
41	95.6	74.4	77.6	76.9	83.4	87.8	83.8	84.0	82.9	74.7	84.1	82.3	81.4	84.2	83.8	79.8	85.7	85.1	90.5	78.0	84.5	27.4	88.0	81.8	80.9	26.4	25.7	25.7	25.6	82.4	25.4	25.7	83.8	86.9	86.7	86.1	88.0	90.9	0.0	0.0		41
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	

1 BPoV Abinanti
2 CMV SH1
3 CPoV HK831F
4 FBoV HK875F
5 GBoV GBoV1
6 HBoV BoV2A-TU-A-114-06
7 HBoV st1
8 HBoV st2
9 HBoV W471
10 PBoV 21ZS-RS
11 PBoV 22
12 PBoV 23
13 PBoV 57AT-HU
14 PBoV 5JS677
15 PBoV 710-3DI-HR
16 PBoV A6
17 PBoV F41
18 PBoV G85-3AT-HU
19 PBoV GD10
20 PBoV GD11
21 PBoV GD6
22 PBoV H18
23 PBoV HN2
24 PBoV PBoV1
25 PBoV PBoV2
26 PBoV procine XJ-12
27 PBoV procine XJ-134
28 PBoV procine XJ-145
29 PBoV procine XJ-27
30 PBoV procine XJ-52
31 PBoV procine XJ-65
32 PBoV procine XJ-79
33 PBoV procine XJ-8
34 PBoV procine XJ-96
35 PBoV SH17N-1
36 PBoV SH17N-2
37 PBoV SH20F
38 PBoV swBoV CH437
39 PBoV SX
40 PPV4 17
41 PPV4 HEN0922-5400