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Sequence Comparison and Phylogenetic Analysis of Several Emerging Porcine Bocaviruses and a Proposal Regarding Nomenclature

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Abstract

The first Bocavirus (bovine parvovirus 1, BPV1) was discovered in calves with diarrhea in 1961. Since then, a series of bocaviruses have emerged in many animals including dogs, humans, swine, gorillas, California sea lions, and cats. These viruses are associated with digestive tract and respiratory tract diseases. Several emerging porcine bocaviruses (PBoVs) have been reported on pig farms in the past four years. However, the nomenclature used to describe these viruses has been confused and irregular. The goal of this study was to compare the sequences of these emerging PBoVs, perform phylogenetic analysis, and present a proposal regarding their nomenclature. Genome sequences of some PBoVs and other bocaviruses were downloaded from the GenBank database. Sequence similarity, sequence mutation, deletion, or insertion, and sequence recombination among reference bocaviruses were obtained using DNAStar software, and its genetic evolution was determined using MEGA 5.1 software. According to the differences in their sequences and genome structure, the nomenclature of PBoVs was proposed and they were divided into four different species (from PBoV1 to PBoV4) and many different genotypes. Among them, both PBoV1 and PBoV2 had two genotypes, PBoV1-a and PBoV1-b and PBoV2-a and PBoV2-b, respectively. PBoV3 included three genotypes, PBoV3-a, PBoV3-b and PBoV3-c, respectively. In addition, for PBoV4, there were at least eight genotypes (PBoV4-a to PBoV4-h) in intraspecies. In conclusion, PBoVs were highly genetically diversified. The proposed nomenclature of PBoVs was used in the present studies, it could help us to better understand and standardize the nomenclature of PBoVs.

Keywords: Emerging porcine bocavirus; Sequence comparison; Phylogenetic analysis; Nomenclature; Proposal

Introduction

Bocaviruses (about 5 kb genome size) are small, non-enveloped, ssDNA viruses within the genus *Bocavirus*, subfamily *Parvovirinae*, family *Parvoviridae* [1]. They cause specific diseases (such as respiratory disease and enteritis) in humans and other animals. Bocaviruses have three open reading frames (ORF), two encoding non-structural proteins (NS1 and NP1), and one structural protein (VP1/VP2). The NP1 protein (the third ORF, ORF3) is characteristic of *Bocavirus* genus, its corresponding genes are located between NS1 gene region and VP1/VP2 gene region [2]. The first bocavirus was discovered in calves with diarrhea in 1961. It was named the HADEN (hemadsorbing enteric) virus of calves [3]. However, it is now called bovine parvovirus 1 (BPV1), which is different from newly discovered BPV2 and BPV3 [4]. About ten years later, minute virus of canines (MVC), also called canine parvovirus type 1 and canine bocavirus, was identified in the rectal specimens of four mature German shepherd dogs [5]. In 2005, an emerging human parvovirus, called human bocavirus (HBoV), similar to BPV1 and MVC in genome structure, was characterized in pooled specimens taken from the respiratory tracts of children with respiratory diseases [6]. Since then, another three species of human bocavirus, HBoV2, HBoV3, and HBoV4, have also been discovered [7-10]. Porcine bocavirus (PBoV) was first described in 2009 in Swedish pigs with postweaning multisystemic wasting syndrome (PMWS) [11]. After that, diversiform PBoVs were reported by scientific researchers from different labs in several countries [12-23]. Its nomenclature was confused and irregular, which might hinder the research of PBoVs. The goal of the present study was to compare the sequences of those emerging porcine bocaviruses, perform phylogenetic analysis, and present a proposal on regarding nomenclature.

Materials and Methods

Reference sequences of porcine and other bocaviruses

In order to select useful materials for this study, genome sequences of some porcine bocaviruses and other bocaviruses described during the same period were downloaded from the GenBank database. The information is presented in (Tables 1 and 2).

Bioinformatic analysis

Bioinformatic software can be used to evaluate sequence similarity among multiple nucleotides and amino acids, sequence mutation, deletions and insertions, sequence recombination, and genetic evolution of viruses, bacteria, and other species. In this study, DNAStar software (Clustal W method) and MEGA 5.1 software (Neighbor-joining method) were used to collect the above information regarding porcine and other bocaviruses. Sequence similarity, sequence mutation, deletion, or insertion, and sequence recombination among reference bocaviruses were obtained using DNAStar software, and its genetic evolution was determined using MEGA 5.1 software.

Results

Genome structure of PBoVs

To better understand genome characterization of different porcine

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Species	Types (Before/Now)	GenBank ID	Genome (bp)	NS1 (bp)	NP1 (bp)	VP1 (bp)	VP2 (bp)	Sources
PBoV1	PBoV-like/PBoV1	HQ223038	4.786	1.911	657	1.872	1.665	China/Serum
	PBoV1/PBoV1	HQ291308	5.267	1.908	657	1.863	1.656	China/Stool
PBoV2	PPV4/PBoV2	GQ387499	5.905	1.797	615	2.187	Uncertain	USA
	PPV4/PBoV2	GQ387500	5.780	1.686	615	2.187	Uncertain	USA
PBoV3	PBoV1/PBoV3	HM053693	5.173	2.112	687	2.118	1.704	China/ Stool
	PBoV2/PBoV3	HM053694	5.186	2.112	693	2.130	1.716	China/Stool
	PBoV2/PBoV3	HQ291309	5.117	2.112	687	2.115	1.701	China/Stool
PBoV4	No/PBoV4	HM053672	2.407	Uncertain	Incomplete	2.055	1.647	China/ Stool
	No/PBoV4	HM053673	2.434	Uncertain	Incomplete	2.043	1.635	China/ Stool
	PBoV3/PBoV4	JF429834	5.278	2.004	681	2.052	1.644	Hong Kong
	PBoV4-1/ PBoV4	JF429835	5.177	2.004	681	2.055	1.647	Hong Kong
	PBoV3/PBoV4	JF512472	5.082	2.403	681	Incomplete	Incomplete	UK/Intestine
	PboV4/PBoV4	JF512473	4.125	2.004	678	Incomplete	Incomplete	UK/Feces
	PBoV3/PBoV4	JF713714	4.710	1.800	681	2.052	1.644	USA/Stool
	PBoV3/PBoV4	JF713715	4.689	1.755	672	2.061	1.653	USA/Stool
	PBoV5/PBoV4	JN831651	5.076	2.004	675	2.055	1.647	China/Stool
	PBoV3C/PBoV4	JN681175	5.235	2.058	675	2.064	1.656	China/Stool

Table 1: Reference genome sequences of porcine bocaviruses in this study.

Species	Genotypes	GenBank ID	Genome (bp)	NS1 (bp)	NP1 (bp)	VP1 (bp)	VP2 (bp)	Sources
BPV1	BPV1	DQ335247	5.515	2.583	642	2.022	1.611	China/Stool
		NC_001540	5.517	2.181	642	2.022	1.611	China/Stool
CBoV	CBoV1	AB518882	5.020	2.325	561	2.112	1.716	Japan
		FJ214110	5.402	2.325	561	2.112	1.716	USA
		FJ899734	5.132	2.325	561	2.112	1.716	China/Stool
	CBoV2	JN648103	5.413	1.947	588	2.139	1.725	USA/Nasal swab
		JQ692589	5.140	1.947	588	2.139	1.725	Hong Kong
HBoV	HBoV1	AB480175	5.299	1.920	660	2.016	1.629	Japan
		DQ000495	5.217	1.920	660	2.016	1.629	Japan
	HBoV2	FJ170278	5.196	1.923	645	2.004	1.617	Pakistan/Stool
		NC_012042	5.196	1.923	645	2.004	1.617	Pakistan/Stool
	HBoV3	EU918736	5.242	1.986	657	2.007	1.620	Australia
		GQ867667	5.161	1.986	657	2.007	1.620	Brazil
	HBoV4	FJ973561	5.104	1.923	645	2.013	1.626	Nigeria/Stool
		NC_012729	5.104	1.923	645	2.013	1.626	Nigeria/Stool
GBoV	GBoV1	HM145750	4.944	2.415	660	2.016	1.629	USA/Stool
		NC_014358	4.944	2.415	660	2.016	1.629	USA/Stool
CslBoV	CslBoV1	JN420360	5.230	2.382	582	2.160	1.746	USA/Feces
	CslBoV2	JN420366	5.283	2.382	582	2.157	1.743	USA/Feces
	CslBoV3	JN420365	5.439	2.409	582	2.160	1.746	USA/Feces
FBoV	FBoV	JQ692585	5.331	2.415	657	2.139	1.719	Hong Kong
		JQ692586	5.179	2.415	657	2.139	1.719	Hong Kong
		JQ692587	5.310	2.415	657	2.139	1.719	Hong Kong

Table 2: Reference genome sequences of other bocaviruses in this study.

bocaviruses, structure maps (Figure 1) were drawn electronically using sequence descriptions given in the GenBank database. PBoV1: Until now, there have been only two nearly complete genomes of PBoV1 in the GenBank database and no fully complete ones, they shared a similar genome structure. ORF1 (encoding NS1 protein) of genotype PBoV1-a had 1,911 NTs and that of genotype PBoV1-b had 1,872 NTs. ORF2 (encoding VP1/VP2 protein) of genotype PBoV1-a had 1,908 NTs and that of PBoV1-b had 1,863 NTs. The first difference lay in a 3-NT insertion or deletion (not shown), and the second difference lay in a 9-NT insertion or deletion (not shown). Both of genotypes had the NP1 genes of the same size (657 NTs) (Figure 1). PBoV1-a and PBoV1-b also had the intervals of the same size nucleotides: there were 185 NTs between the area downstream of NS1 and the area upstream and there were 52 NTs between the area downstream of NP1 and the area upstream (not shown).

PBoV2 has been reported in some countries, including the USA, China, Romania, Hungary, and Cameroon [13,14; 24-26]. Descriptions of its genome are limited. Generally, the isolates from the USA and China are divided into two different genotypes, PBoV2-a and PBoV2-b, respectively. ORF2 (encoding VP1/VP2 protein) and ORF3 (encoding NP1 protein) of PBoV2-a and PBoV2-b had similar structures (2, 187 NTs for VP1/VP2 genes and 615 NTs for NP1 genes) (Figure 1). They also had intervals of the same length. There were 149 NTs between the upstream and downstream areas of PBoV2-a NS1 and 263 NTs between the upstream and downstream areas of PBoV2-b NP1 (not shown). PBoV2-b differed from other

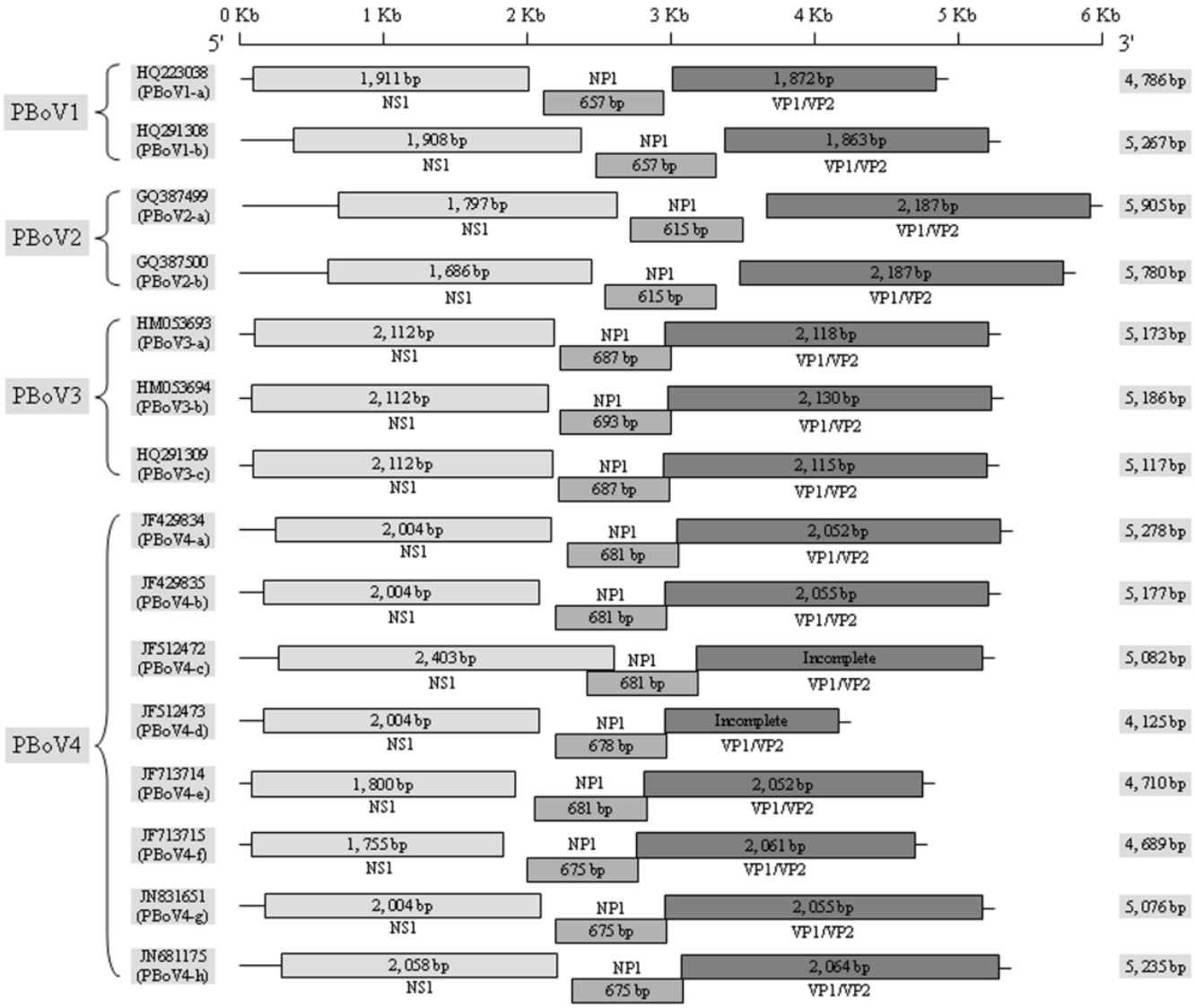


Figure 1: Genome structure of different porcine bocaviruses.

isolates in that it had a 111 NT deletion from the area upstream of the NS1 gene (not shown).

PBoV3 was discovered using viral metagenomic methods [12,27]. At present, the isolates of PBoV3 can be divided into three different genotypes, PBoV3-a, PBoV3-b, and PBoV3-c. The NS1 genes of these three genotypes are the same size (2,112 NTs) (Figure 1). In comparison to PBoV3-a (687 NTs) and PBoV3-c (687 NTs), there was a discontinuous 6-NT (3+3) deletion in the NP1 genes of PBoV3-b (693 NTs) (not shown). The VP1/VP2 genes differ from those of PBoV3-b (2,130 NTs) in a discontinuous 12 NT (9+3) deletion and 15 NT (12+3) deletion (not shown), leaving length of these VP1/VP2 genes were 2,118 NTs and 2,115 NTs, respectively (Figure 1). PBoV3-a and PBoV3-c had intervals of the same size (72 NTs) between the area downstream of NS1 and the area upstream of NP1. They also had the same number of overlapping nucleotides (17 NTs) between the area downstream of NP1 and the area upstream of VP1/VP2. However, PBoV3-b had the same number of overlapping nucleotides (17 NTs) as PBoV3-a and PBoV3-c between the area downstream of NP1 and the area upstream of VP1/VP2, but it also had a different number of interval nucleotides (69 NTs) between the area downstream of NS1 and the area upstream of NP1 (not shown).

The species PBoV4 can be divided into eight genotypes by the differences in its genome structure, PBoV4-a to PBoV4-h. PBoV4-a, PBoV4-b, PBoV4-d, and PBoV4-g had NS1 genes of 2,004 NT in length, but the others had NS1 genes of different lengths: 2,403 NT for PBoV4-c, 1,800 NTs for PBoV4-e, 1,755 NT for PBoV4-f, and 2,058 NT for PBoV4-h. PBoV4-a, PBoV4-b, PBoV4-c, and PBoV4-e had NP1 genes of 681 NT in length, and PBoV4-f, PBoV4-g, and PBoV4-h had NP1 genes of 675 NT in length. However, the length of the NP1 gene of PBoV4-d was 678 NT. PBoV4-b, PBoV4-c, and PBoV4-g had VP1/VP2 genes of 2,055 NT in length, PBoV4-a and PBoV4-c had VP1/VP2 genes of 2,052 NT in length, and PBoV4-d and PBoV4-f had VP1/VP2 genes that differed from each other (Figure 1). Almost all PBoV4 genotypes had 9 overlapping sequences between NP1 and VP1/VP2. However, there were intervals of many different sizes between NS1 and NP1. PBoV4-a and PBoV4-e both had intervals of 121 NT. PBoV4-b and PBoV4-d both

had intervals of 124 NT. PBoV4-f and PBoV4-g had intervals of 129 NT. PBoV4-h had the shortest interval (107 NT). Notably, the NS1 and NP1 of PBoV4-c overlapped by 276 NT (not shown).

Nucleotide similarity of different protein-encoding regions of PBoVs

NS1 region: As shown in (Figure 1), the size of NS1 among PBoVs varied from 1,755 NT to 2403 NT. Even if the viral strains belonged to the same species, there were still differences in the size of NS1. Generally, nucleotide similarity of the NS1 region varied from 6.8% to 99.8% (Table 3). HQ223038 and HQ291308 both had 77.4% nucleotide similarity to PBoV1. For PBoV2, the truncated NS1 (lacking 111 NTs) of GQ387499 and the complete NS1 of GQ387500 had 99.8% nucleotide similarity. For PBoV3, the NS1 genes of HM053693, HM053694, and HQ291309 were 2112 bp in size, but their homologies varied from 93.8% to 94.3%. For PBoV4, the nucleotide similarity of those strains varied between 79.1% and 96.8%.

NP1 region: The NP1 genes of PBoV1-4 were no more than 700 nucleotides in size. However, there were considerable changes in nucleotide similarity, from 3.1% to 99.7% (Table 4). For PBoV1, HQ223038 and HQ291308 had 99.2% nucleotide similarity, which was higher than that of NS1. For PBoV2, like NS1, the NP1 of GQ387499 and GQ387500 had considerable nucleotide similarity (99.7%). For PBoV3, HM053693, HM053694, and HQ291309 had less nucleotide similarity (from 91.6% to 93.7%) than NS1 did. For PBoV4, there was at least 95% nucleotide similarity between JF429834, JF429835, JF512472, and JF713714. JF713715 and JN831651 also had 91.7% nucleotide similarity. For PBoV4, they had about 80% nucleotide similarity.

VP1/VP2 region: For the VP1/VP2 region, inter-species nucleotide similarity was low, ranging from 0.8% to 58.6%. More nucleotide similarity (from 93.1% to 99.8%) was detected among intra-species (PBoV1, PBoV2, and PBoV3) than PBoV4 (from 75.3% to 88.7%) (Table 5).

Phylogenetic analysis of different protein-encoding regions of PBoVs

A phylogenetic tree of NS1 was constructed based on two PBoV1

	HQ223038	HQ291308	GQ387499	GQ387500	HM053693	HM053694	HQ291309	HM053672	HM053673	JF429834	JF429835	JF512472	JF512473	JF713714	JF713715	JN831651	JN681175
HQ223038	100	77.4	13.4	14.2	41.1	41.1	40.7	—[a]	—[a]	27.1	27.2	27.6	25.1	24.3	25.0	26.9	22.7
HQ291308		100	14.8	15.7	41.0	41.0	41.7	—[a]	—[a]	23.4	23.2	23.4	28.0	25.1	25.7	27.7	27.3
GQ387499			100	99.8	8.8	8.9	8.9	—[a]	—[a]	7.1	6.8	13.0	13.7	7.8	7.9	12.9	13.4
GQ387500				100	9.4	9.4	9.4	—[a]	—[a]	7.6	7.3	13.8	14.6	8.2	8.2	13.7	14.3
HM053693					100	94.3	93.8	—[a]	—[a]	46.4	45.7	44.3	46.8	51.7	53.3	48.4	47.7
HM053694						100	94.2	—[a]	—[a]	46.3	46.2	44.7	26.8	51.9	53.2	34.8	48.1
HQ291309							100	—[a]	—[a]	45.1	45.6	42.8	26.3	50.2	51.7	33.6	45.7
HM053672								100	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
HM053673									100	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
JF429834										100	94.2	96.8	82.0	95.8	94.6	81.5	81.5
JF429835											100	93.6	82.4	95.2	93.0	81.4	80.8
JF512472												100	81.5	95.6	93.7	80.7	80.6
JF512473													100	83.0	84.4	86.6	80.2
JF713714														100	95.2	81.7	81.2
JF713715															100	83.8	82.1
JN831651																100	79.2
JN681175																	100

Note: [a]The NS1 region of HM053672 and HM053672 was not available, so its nucleotide homology with other PBoV strains was not listed.

Table 3: Nucleotide homology (%) of NS1 regions of PBoV1-4.

	HQ223038	HQ291308	GQ387499	GQ387500	HM053693	HM053694	HQ291309	HM053672	HM053673	JF429834	JF429835	JF512472	JF512473	JF713714	JF713715	JN831651	JN681175
HQ223038	100	99.2	3.7	3.7	36.2	35.3	35.3	—[a]	—[a]	24.5	24.7	23.0	24.2	24.4	29.8	25.3	22.7
HQ291308		100	3.1	3.1	36.1	35.2	35.2	—[a]	—[a]	24.7	24.8	23.1	24.5	24.5	29.7	25.1	23.0
GQ387499			100	99.7	4.1	5.7	4.7	—[a]	—[a]	4.7	3.1	4.6	4.6	4.7	4.7	4.6	3.9
GQ387500				100	2.0	5.7	4.2	—[a]	—[a]	4.7	3.1	4.6	4.6	4.7	4.7	4.6	3.9
HM053693					100	92.7	93.7	—[a]	—[a]	32.9	15.3	14.7	28.6	28.0	28.7	33.3	11.0
HM053694						100	91.6	—[a]	—[a]	28.8	28.6	28.6	24.6	28.9	29.0	25.2	23.9
HQ291309							100	—[a]	—[a]	28.6	28.5	28.0	24.0	28.3	29.0	24.6	24.1
HM053672								100	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
HM053673									100	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
JF429834										100	98.5	96.3	83.9	98.4	82.4	83.1	80.3
JF429835											100	96.5	85.0	98.8	82.9	83.9	81.5
JF512472												100	84.7	96.2	81.7	83.6	80.4
JF512473													100	84.5	86.0	86.8	82.1
JF713714														100	83.2	84.0	80.9
JF713715															100	91.7	79.6
JN831651																100	78.5
JN681175																	100

Table 4: Nucleotide homology (%) of NP1 regions of PBoV1-4.

	HQ223038	HQ291308	GQ387499	GQ387500	HM053693	HM053694	HQ291309	HM053672	HM053673	JF429834	JF429835	JF512472	JF512473	JF713714	JF713715	JN831651	JN681175
HQ223038	100	99.6	2.3	2.3	50.1	51.3	52.6	19.4	50.3	49.9	19.3	—[a]	—[a]	52.5	50.1	50.5	16.4
HQ291308		100	2.3	2.3	50.0	51.5	52.8	50.9	51.2	50.1	19.5	—[a]	—[a]	52.7	50.7	50.8	47.2
GQ387499			100	99.8	1.1	1.7	0.9	2.5	2.9	0.9	0.8	—[a]	—[a]	1.4	1.7	3.0	1.2
GQ387500				100	1.1	1.7	0.9	2.5	2.9	0.9	0.8	—[a]	—[a]	1.4	1.0	3.0	1.2
HM053693					100	93.1	96.0	58.0	57.8	57.6	56.9	—[a]	—[a]	57.3	56.9	57.7	58.6
HM053694						100	93.1	58.4	58.1	57.8	57.9	—[a]	—[a]	58.3	56.9	57.4	59.3
HQ291309							100	57.8	58.2	57.5	57.2	—[a]	—[a]	56.9	56.1	57.4	58.6
HM053672								100	94.5	82.4	86.5	—[a]	—[a]	78.7	80.4	90.0	83.6
HM053673									100	80.6	86.0	—[a]	—[a]	78.0	80.1	92.4	84.4
JF429834										100	80.8	—[a]	—[a]	80.4	88.7	79.4	77.1
JF429835											100	—[a]	—[a]	79.5	81.8	87.3	84.3
JF512472												100	—[a]	—[a]	—[a]	—[a]	—[a]
JF512473													100	—[a]	—[a]	—[a]	—[a]
JF713714														100	81.0	78.2	75.3
JF713715															100	79.2	77.6
JN831651																100	85.5
JN681175																	100

Note: [a] The VP1/VP2 region of JF512472 and JF512473 was not complete, so its nucleotide homology with other PBoV strains was not listed.

Table 5: Nucleotide homology (%) of VP1/VP2 regions of PBoV1-4.

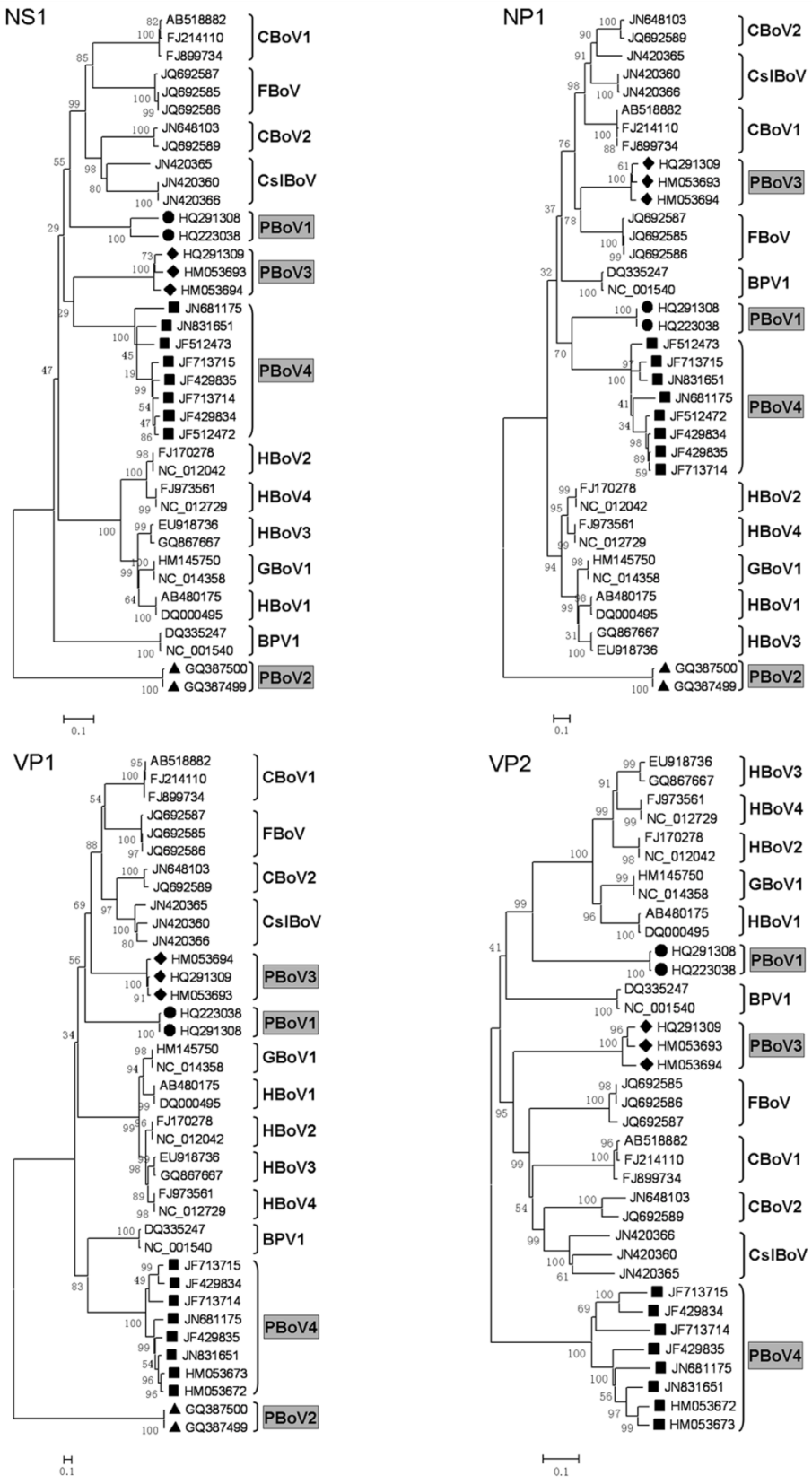
strains, 2 PBoV2 strains, 3 PBoV3 strains, 8 PBoV4 strains, and another 25 bocavirus strains (Figure 2). PBoV1, PBoV3, and PBoV4 were clustered in the branch with CBoV1, CBoV2, FBoV, and CslBoV. However, PBoV2 was divided into one separate branch, which had farthest genetic distances from the other bocaviruses (Figure 2).With respect to NP1, PBoV1 and PBoV4 had close genetic relationships and were clustered in the same branch. PBoV3 and FBoV were placed on the same branch. Like NS1, PBoV2 was placed far from other viral species (Figure 2).With respect to VP1, PBoV1 and PBoV3 had less genetic differences from CBoV1, CBoV2, FBoV, and CslBoV and were placed on one big branch. PBoV4 had the close genetic relationship to BPV1. PBoV2 was far from other bocaviruses in the phylogenetic tree (Figure 2). With respect to VP2, PBoV1 was close to HBoV1-4, GBoV1, and BPV1. Similarly, PBoV3 was not far from CBoV1, CBoV2, FBoV, or CslBoV. PBoV4 was far from other bocaviruses (Figure 2).

Discussion and Nomenclature of PBoVs

PBoV1 was discovered in 2009, before any of the others [11]. This is why it was named PBoV1 [18,23]. Shan et al. [18] found PBoV1 circulating in China. Then, it had also been found in Romania, Uganda, and Cameroon [26,28,29]. Due to the low level of nucleotide similarity (especially in the NS1 region) and genome characterization among species, PBoV1 should be divided into two genotypes, PBoV1-a and PBoV1-b (Figure 1). In 2010, Cheung et al. [13] identified a novel porcine parvovirus different from previous parvoviruses (PPV1, PPV2, and PPV3). This virus was tentatively named PPV4. However, its genome structure and RNA profile showed it had the characteristics of bocaviruses, herein, so it was renamed PBoV2 [23,30]. Considering the major differences within the PBoV2 genome, it should include two genotypes. PBoV2-a and PBoV2-b, the second of which lacks 111 NT in its NS1 region (Figure 1).Two Chinese research teams detected two novel bocaviruses almost simultaneously. They were named PBoV1 and PBoV2 [12,18]. Based on the sequence comparison and phylogenetic analysis described herein, these two strains are named PBoV3. PBoV3 should contain three genotypes, PBoV3-a, PBoV3-b, and PBoV3-c (Figure 1). However, there was considerable nucleotide similarity in different coding regions (93.8% to 94.3% for NS1, 91.6% to 93.7% for NP1, and 93.1% to 96% for VP1/VP2) (Tables 3-5). PBoV3 and non-PBoV3 (present PBoV4) were found and reported at about the same time [12]. Many PBoV4-like genome sequences have been published [15-17,19-20]. These PBoV4-like strains could be considered as members of the PBoV4 species. Based on sequence differences, PBoV4 can be divided into eight different genotypes (PBoV4-a and PBoV4-h) (Figure 1). Moreover, to our knowledge, at present, porcine bocaviruses were cultured poorly *in vitro*. Therefore, it is very difficult to us to master the pathogenicity for every PBoV species. However, some epidemiological studies showed that PBoV1, PBoV2 and PBoV4 had higher prevalence in diseased pig herds than PBoV3 [11-14,22,23]. Whether the sequence diversities of viral genes (NS1, VP1/VP2) could have any impact on the virulence or infectivity of different virus species should be further studied. According to the present literatures, All the four PBoV species were reported in Europe (such as Sweden, Hungary), Asia (such as China, South Korea, Hong Kong), North America, Africa (such as Uganda, Cameroon) [31]. It suggested that PBoVs lacked of the significant geographical prevalence, which was associated with frequent international trading of live pigs and pork products. Meanwhile, there were few PBoV reports in Australia, South America, Southeast Asia, the Middle East countries. The reason was that no research team did epidemiological survey in those regions. For the origin of every PBoV species, the mystery was still not uncovered.

Conclusion

The current criteria for classification of bocaviruses issued by the International Committee on Taxonomy of Viruses (ICTV) defines separate species as a group of individuals with less than 95% homologous non-structural gene DNA sequence (http://www.ictvdb.org/). However, the NS1 genes of PBoVs divide them into ten species, but their NP1 genes only divide them into nine. Recently, VP1-based classification has also been proposed. PBoVs were divided into three groups: group 1 or PBoV1 (represented by PBo-likeV), group 2 or PBoV2 (PBoV1/2-CHN and PBoV2-A6), and group 3 or PBoV3 (PBoV3/4-UK, PBoV3/4-HK, and PBoV3C) [20]. However, here, we added PPV4 (boca-like virus) to the PBoV family. According to the present proposal, which is based on genome comparison and phylogenetic analysis, PBoVs can be divided into four different species, PBoV1 (represented by PBo-likeV-SWE and PBoV1-CHN), PBoV2 (PPV4-USA), PBoV3 (PBoV1/2-CHN and PBoV2-A6-CHN), and PBoV4 (PBoV3/4-UK, PBoV3/4-HK, PBoV3-USA, PBoV3C-CHN, and PBoV5-CHN). Further classification showed that these four species also contained 2-8 different genotypes, respectively. In summary, it is here suggested that PBoVs are highly genetically diversified. The proposed nomenclature of PBoVs was used in the present studies, and it may facilitate better understanding of PBoVs. If nothing else, it may facilitate the development of a standardized nomenclature system.



Note: Phylogenetic tree was constructed using neighbor-joining method (MEGA 5.1 software). Bootstrap replications and genetic distances were set by 1000 and 0.1.

Figure 2: Phylogenetic analysis of NS1, NP1, VP1 and VP2 genes for porcine bocaviruses and other reference bocavirus.

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References

1. Manteufel J, Truyen U (2008) Animal bocaviruses: a brief review. Intervirology 51: 328-334.
2. Lau SK, Woo PC, Yeung HC, Teng JL, Wu Y, et al. (2012) Identification and characterization of bocaviruses in cats and dogs reveals a novel feline bocavirus and a novel genetic group of canine bocavirus. J Gen Virol 93: 1573-1582.
3. Abinanti FR, Warfield MS (1961) Recovery of a hemadsorbing virus (HADEN) from the gastrointestinal tract of calves. Virology 14: 288-289.
4. Allander T, Emerson SU, Engle RE, Purcell RH, Bukh J (2001) A virus discovery method incorporating DNase treatment and its application to the identification of two bovine parvovirus species. Proc Natl Acad Sci USA 98: 11609-11614.
5. Binn LN, Lazar EC, Eddy GA, Kajima M (1970) Recovery and characterization of a minute virus of canines. Infect Immun 1: 503-508.
6. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, et al. (2005) Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA 102: 12891-12896.
7. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM (2009) A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Patho 5: e1000391.
8. Kapoor A, Slikas E, Simmonds P, Chieochansin T, Naeem A, et al. (2009) A newly identified bocavirus species in human stool. J Infect Dis 199: 196-200.
9. Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L, et al. (2010) Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis 201: 1633-1643.
10. Santos N, Peret TC, Humphrey CD, Albuquerque MC, Silva RC, et al. (2010) Human bocavirus species 2 and 3 in Brazil. J Clin Virol 48: 127-130.
11. Blomström AL, Belák S, Fossum C, McKillen J, Allan G, et al. (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.
12. Cheng WX, Li JS, Huang CP, Yao DP, Liu N, et al. (2010) Identification and nearly full-length genome characterization of novel porcine bocaviruses. PLoS One 5: e13583.
13. Cheung AK, Wu G, Wang D, Bayles DO, Lager KM, et al. (2010) Identification and molecular cloning of a novel porcine parvovirus. Arch. Virol 155: 801-806.
14. Huang L, Zhai SL, Cheung AK, Zhang HB, Long JX, et al. (2010) Detection of a novel porcine parvovirus, PPV4, in Chinese swine herds. Virol J 7: 333.
15. Lau SK, Woo PC, Yip CC, Li KS, Fu CT, et al. (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. J Gen Virol 92: 2047-2059.
16. Li B, Ma J, Xiao S, Fang L, Zeng S, et al. (2012) Complete genome sequence of a novel species of Porcine Bocavirus, PBoV5. J Virol 86: 1286-1287.
17. McKillen J, McNeilly F, Duffy C, McMenamy M, McNair I, et al. (2011) Isolation in cell cultures and initial characterisation of two novel bocavirus species from swine in Northern Ireland. Vet Microbiol 152: 39-45.
18. Shan T, Lan D, Li L, Wang C, Cui L, et al. (2011) Genomic characterization and high prevalence of bocaviruses in swine. PLoS One 6: e17292.
19. Shan T, Li L, Simmonds P, Wang C, Moeser A, et al. (2011) The fecal virome of pigs on a high-density farm. J Virol 85: 11697-11708.
20. Yang WZ, Yu JM, Li JS, Cheng WX, Huang CP, et al. (2012) Genome characterization of a novel porcine bocavirus. Arch Virol 157: 2125-2132.
21. Zeng S, Wang D, Fang L, Ma J, Song T, et al.(2011) Complete coding sequences and phylogenetic analysis of porcine bocavirus. J Gen Virol 92: 784-788.
22. Zhai S, Yue C, Wei Z, Long J, Ran D, et al. (2010) High prevalence of a novel porcine bocavirus in weanling piglets with respiratory tract symptoms in China. Arch Virol 155: 1313-1317.
23. Zhang HB, Huang L, Liu YJ, Lin T, Sun CQ, et al. (2011) Porcine bocaviruses: genetic analysis and prevalence in Chinese swine population. Epidemiol Infect 139: 1581-1586.
24. Blomström AL, Ståhl K, Masembe C, Okoth E, Okurut AR, et al. (2012) Viral metagenomic analysis of bushpigs (Potamochoerus larvatus) in Uganda identifies novel variants of Porcine parvovirus 4 and Torque teno sus virus 1 and 2. Virol J 9: 192.
25. Cságola A, Lőrincz M, Cadar D, Tombácz K, Biksi I, et al. (2012) Detection, prevalence and analysis of emerging porcine parvovirus infections. Arch Virol 157: 1003-1010.
26. Ndze VN, Cadar D, Cságola A, Kisfali P, Kovács E, et al. (2013) Detection of novel porcine bocaviruses in fecal samples of asymptomatic pigs in Cameroon. Infect, Genet & Evol 17: 277-282.
27. Shan TL, Cui L, Dai XQ, Guo W, Shang XG, et al. (2010) Sequence analysis of an isolate of minute virus of canines in China reveals the closed association with bocavirus. Mol Biol Rep 37: 2817-2820.
28. 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.
29. Cadar D, Cságola A, Lorincz M, Tombácz K, Kiss T, et al. (2011) Genetic detection and analysis of porcine bocavirus type 1 (PoBoV1) in European wild boar (Sus scrofa). Virus Genes 43: 376-379.
30. Cheung AK, Long JX, Huang L, Yuan SS (2011) The RNA profile of porcine parvovirus 4, a boca-like virus, is unique among the parvoviruses. Arch Virol 156: 2071-2078.
31. Zhou F, Sun H, Wang Y (2014) Porcine bocavirus: achievements in the past five years. Viruses 6: 4946-4960.