


# Heterogeneity of *Pestivirus* Species in Asia

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## Abstract

*Pestivirus* are responsible for cosmopolitan diseases affecting cattle, pigs and other ruminants, presenting a wide range of clinical manifestations, with relevant impact on zootechnic production. Understanding genomic characteristic and virus taxonomy is fundamental in order to sustain control and prophylactic programs. Given the recent various studies reporting a relatively high number of new strains, in particular from Asian countries, in the present study, six hundred-fifty-one genomic sequences have been considered applying the palindromic nucleotide substitutions method for genotyping. Based on the secondary structure analysis of the 5' untranslated region of RNA, sequence characteristics among Asian genomic clusters within the different *Pestivirus* species suggested geographic segregation and occurrence of micro-evolutionary steps in the genus evolutionary history. This aspect was particularly evident in atypical sequences originated from China or Turkey, indicating risk of diffusion by animals and products trade or contamination of biological products as bovine calf serum, with potential diagnostic and control difficulties.

## Keywords

Asia, Genotypes, *Pestivirus*, Secondary Structure

## 1. Introduction

Species of the genus *Pestivirus* of the family *Flaviviridae* [1] are responsible for cosmopolitan disease affecting cattle pig and other ruminants, presenting a wide range of clinical manifestations, with relevant impact on zootechnic production. Different reports described atypical variants within the bovine viral diarrhoea virus type 1 (BVDV-1) (*Pestivirus* A), characterized by genetic traits different

from most common previously reported strains, in particular from Asian countries [2]-[10]. Similarly, Asian atypical sequences have been reported in other *Pestivirus* species as BVDV-3 (*Pestivirus H*) and Classical swine fever virus (CSFV) (*Pestivirus C*) [11] [12] [13]. Taking into account that a certain confusion in the nomenclature of the increasing number of *Pestivirus* genetic variants was evident and it is still a matter of concern, indicating that the need for harmonization [14] [15] [16], and the utility to have an alternative evaluation method are always interesting in any discipline, the palindromic nucleotide substitutions (PNS) method [17] [18] was proposed for an analytical procedure not limited to primary structure analysis but extended to secondary structure, focusing on strategic genomic sequences corresponding to the internal ribosome entry site (IRES), in the 5' untranslated region (UTR), responsible for translational, transcriptional and replication events in pestiviruses [19]. In order to evaluate more exhaustively the taxonomical segregation of the genus *Pestivirus* in Asian countries, the 5'-UTR genomic sequences of the BVDV-1, BVDV-2 (*Pestivirus B*), BVDV-3, Border Disease virus (BDV) (*Pestivirus D*) and CSFV species were analyzed and compared applying the PNS genotyping method, encompassing all *Pestivirus* species circulating in domestic animals in Asia and excluding the pestiviruses from chiropters.

## 2. Material and Methods

In order to determine genotypic variations in the BVDV-1, BVDV-2, BVDV-3, BDV and CSFV species, the 5'-UTR genomic region of six hundred-fifty-one *Pestivirus* strains, have been analyzed for numerical taxonomy. The virus nucleotide sequences, with different geographical origin, primarily of Asian origin, from different host species or contaminants of biological products, were obtained from the DDBL/EMBL/GenBank DNA database or provided by authors (Table 1). Strains from Europe and Americas have been considered as reference of genetic variants and complete comparison of strain sequences of BVDV-2, BVDV-3 and CSFV species. The majority of the tested virus sequences were originated from strains isolated from cattle (*Bos taurus*) from China (n 131). Other bovine strains originated from various other countries from Middle East, Indian subcontinent, Far East and Austral Asia (Turkey, Iran, Bangladesh, India, Thailand, Japan, South Korea, Australia and New Zealand) (n 69), and 89 were from Europe and Americas. Sixteen strains were isolated from buffalo (*Bubalus bubalis*) in China. Strains isolated from domestic Bactrian camels (*Camelus bactrianus*) and yaks (*Bos grunniens*) have also been reported from China (n 60). Seventeen strains were reported from Zebu (*Bos indicus*) in India, 116 strains were isolated from domestic and wild suids, pigs (*Sus scrofa domesticus*) and wild boar (*Sus scrofa*), 63 were from small ruminants, sheep (*Ovis aries*), goat (*Capra hircus*) and black goat (*Capra aegagrus hircus*). Three strains were from Alpine chamois (*Rupicapra rupicapra*), Pyrenean chamois (*Rupicapra pyrenaica*) and Reindeer (*Rangifer tarandus*), and 3 from Japanese serow (*Capricornis crispus*). A single

**Table 1.** List of *Pestivirus* strains of BVDV-1 (n = 296), BVDV-2 (n = 128), BVDV-3 (n = 62), BDV (n = 37), *Pestivirus I* (n = 2) and CSFV (n = 126) species evaluated according to palindromic secondary structure characteristics at the RNA 5'-UTR (PNS method). Nomenclature of identified genotypes is based on divergence in the genus. Clustering according to primary structure analysis by depositors is indicated under parenthesis. ND: not determined.

Species/Genotype	Strain	Origin	Country	Accession	Reference
BVDV-1a	0192	Contaminant	Japan	D31799	[118]
BVDV-1a	12	Cattle	Japan	D26051	[117]
BVDV-1a	2L91	Contaminant	Japan	D31800	[118]
BVDV-1a	A014	Contaminant	Japan	D31801	[118]
BVDV-1a (A)	B2-175/AU	Contaminant	Australia	JN967708	[29]
BVDV-1a (A)	BJ1308	Cattle	China	KF925517	[154]
BVDV-1a	C009T	Contaminant	Japan	D31802	[118]
BVDV-1a (A)	E3-177/NZ	Contaminant	New Zealand	JN967722	[29]
BVDV-1a	FU411	Contaminant	Japan	D31804	[115]
BVDV-1a (A)	H1-181/AU	Contaminant	Australia	JN967730	[29]
BVDV-1a	H503	Contaminant	Japan	AB008841	[112]
BVDV-1a	HC725	Contaminant	Japan	D31805	[118]
BVDV-1a	HE726	Contaminant	Japan	D31806	[118]
BVDV-1a	HE728	Contaminant	Japan	D31808	[118]
BVDV-1a	HH	Contaminant	Japan	D50818	[30]
BVDV-1a (A)	HN01	Cattle	China	JX878887	[5]
BVDV-1a	IQ19A	Contaminant	Japan	D31812	[118]
BVDV-1a (A)	isolate 1	Bactrian camel	China	JX276538	[31]
BVDV-1a (A)	isolate 2	Bactrian camel	China	JX276539	[31]
BVDV-1a	JE	Contaminant	Japan	D26611	[115]
BVDV-1a	KQ25A	Contaminant	Japan	D31809	[118]
BVDV-1a	KQ25B	Contaminant	Japan	D31810	[118]
BVDV-1a (J)	KS86-1cp	Cattle	Japan	AB078952	[138]
BVDV-1a	L1305	Cattle	New Zealand	AF026781	Vilcek <i>et al.</i> , unpublsh
BVDV-1a	LQ28A	Contaminant	Japan	D31811	[118]
BVDV-1a	MMR-K	Contaminant	Japan	D26050	[117]
BVDV-1a (A)	Nose	Cattle	Japan	AB019670	[44]
BVDV-1a (A)	SH1060	Pig	China	JN248741	[32]
BVDV-1a	TK-87-2	Cattle	Japan	AB019669	[44]
BVDV-1a (C)	Trangie Y546	Cattle	Australia	AF049222	Gu <i>et al.</i> , unpublsh
BVDV-1a	TY CP/91	Cattle	Japan	AB042670	[90]
BVDV-1.b1 (B)	BJ09_04	Cattle	China	HQ116537	Zhang <i>et al.</i> , unpublsh
BVDV-1b1	CV-1	Contaminant	Japan	D50815	[30]
BVDV-1b1	FS720	Contaminant	Japan	D31803	[118]
BVDV-1b1	HeLa	Contaminant	Japan	D50819	[30]
BVDV-1.b1 (B)	JS-05059	Cattle	China	KJ578849	[2]
BVDV-1b1	MDBK	Contaminant	Japan	D50820	[30]
BVDV-1b1	MDCK	Contaminant	Japan	D50821	[30]
BVDV-1b1	MOLT-4	Contaminant	Japan	D50822	[30]
BVDV-1b1	Mumps	Contaminant	Japan	D26049	[117]
BVDV-1b1	U937	Contaminant	Japan	D50823	[30]
BVDV-1b1	Vero	Contaminant	Japan	D50824	[30]
BVDV-1b1	Wi-38	Contaminant	Japan	D50825	[30]
BVDV-1b1	WiDr	Contaminant	Japan	D50826	[30]
BVDV-1b1	Ind 446	Cattle	India	AY279087	[131]
BVDV-1b1	IndS1166	Cattle	India	AY278460	[131]
BVDV-1b1	IndS1168	Cattle	India	AY279086	[131]

## Continued

BVDV-1b1	IndS1170	Cattle	India	AY279526	[131]
BVDV-1b1	IndS1171	Cattle	India	AY279527	[131]
BVDV-1b1	IndS1181	Cattle	India	AY279528	[131]
BVDV-1b1	IndS1222	Cattle	India	AY278459	[131]
BVDV-1b1	IndS1455	Cattle	India	AY278461	[131]
BVDV-1b2	IndMDV18697/12	Zebu	India	KM201317	[13]
BVDV-1.b2 (B)	HB-0134	Cattle	China	KJ578829	[2]
BVDV-1.b2 (B)	HB-0258	Cattle	China	KJ578827	[2]
BVDV-1.b2 (B)	HB-0288	Cattle	China	KJ578826	[2]
BVDV-1.b2 (B)	HB-060085	Cattle	China	KJ578825	[2]
BVDV-1.b2 (B)	HB-0899	Cattle	China	KJ578833	[2]
BVDV-1.b2 (B)	HB-090219	Cattle	China	KJ578828	[2]
BVDV-1b2 (B)	isolate 12	Bactrian camel	China	JX276549	[31]
BVDV-1b2	KA-91	Cattle	Japan	AB019684	[44]
BVDV-1.b2 (B)	LN311-15	Cattle	China	KJ578799	[2]
BVDV-1.b2 (B)	LN311-17	Cattle	China	KJ578798	[2]
BVDV-1.b2 (B)	LN311-25	Cattle	China	KJ578797	[2]
BVDV-1.b2 (B)	LN311-27	Cattle	China	KJ578796	[2]
BVDV-1.b2 (B)	LN311-34	Cattle	China	KJ578795	[2]
BVDV-1.b2 (B)	LN313-15	Cattle	China	KJ578800	[2]
BVDV-1.b2 (B)	LN314-19	Cattle	China	KJ578801	[2]
BVDV-1.b2 (B)	QHMY-21	Yak	China	KJ578902	[2]
BVDV-1.b2 (B)	QHMY-N5	Yak	China	KJ578900	[2]
BVDV-1.b2 (B)	QHMY-N6	Yak	China	KJ578901	[2]
BVDV-1.b2 (B)	QHQL-126	Yak	China	KJ578880	[2]
BVDV-1.b2 (B)	QHQL-219	Yak	China	KJ578890	[2]
BVDV-1.b2 (B)	QHQL-271	Yak	China	KJ578891	[2]
BVDV-1.b2 (B)	QHQL-292	Yak	China	KJ578883	[2]
BVDV-1.b2 (B)	QHQL-295	Yak	China	KJ578886	[2]
BVDV-1.b2 (B)	QHQL-297	Yak	China	KJ578892	[2]
BVDV-1.b2 (B)	QHQL-299	Yak	China	KJ578893	[2]
BVDV-1.b2 (B)	QHQL-311	Yak	China	KJ578873	[2]
BVDV-1.b2 (B)	QHQL-313	Yak	China	KJ578874	[2]
BVDV-1.b2 (B)	QHQL-321	Yak	China	KJ578877	[2]
BVDV-1.b2 (B)	QHQL-328	Yak	China	KJ578875	[2]
BVDV-1.b2 (B)	QHQL-336	Yak	China	KJ578887	[2]
BVDV-1.b2 (B)	QHQL-337	Yak	China	KJ578876	[2]
BVDV-1.b2 (B)	QHQL-340	Yak	China	KJ578878	[2]
BVDV-1.b2 (B)	QHQL-345	Yak	China	KJ578879	[2]
BVDV-1.b2 (B)	QHQL-385	Yak	China	KJ578888	[2]
BVDV-1.b2 (B)	QHQL-405	Yak	China	KJ578889	[2]
BVDV-1.b2 (B)	QHTJ-17	Yak	China	KJ578896	[2]
BVDV-1.b2 (B)	QHTJ-291	Yak	China	KJ578894	[2]
BVDV-1.b2 (B)	QHTJ-L332	Yak	China	KJ578895	[2]
BVDV-1.b2 (B)	QHTJ-N303868	Yak	China	KJ578897	[2]
BVDV-1.b2 (B)	QHTJ-N304810	Yak	China	KJ578898	[2]
BVDV-1.b2 (B)	XZ-117	Yak	China	KJ578904	[2]
BVDV-1.b2 (B)	XZ-70	Yak	China	KJ578911	[2]
BVDV-1.3 (D)	10JJ-SKR	Cattle	South Korea	KC757383	[123]
BVDV-1.3 (D)	B1-1/AU	Contaminant	Australia	JN967745	[29]
BVDV-1.3 (D)	BJ1023	Cattle	China	KF925509	[154]



## Continued

BVDV-1.3 (D)	BJ1120	Cattle	China	KF925510	[154]
BVDV-1.3 (D)	BJ1201	Cattle	China	KF925513	[154]
BVDV-1.3	BRU*0615	Contaminant	Japan	AB008837	[112]
BVDV-1.3 (D)	cell-con-1	Cattle	China	KC695816	Gao <i>et al.</i> , unpubl
BVDV-1.3 (D)	IR-Isfahan-10	Cattle	Iran	LC053999	Shapouri <i>et al.</i> , unpubl
BVDV-1.3 (D)	OK1(CA)NCP03	Cattle	Japan	AB359927	[28]
BVDV-1.3	Kamoshika-3	Serow	Japan	AB259693	[63]
BVDV-1.3	Kamoshika-22	Serow	Japan	AB259694	[63]
BVDV-1.3	Kamoshika-29	Serow	Japan	AB259695	[63]
BVDV-1.4	438/02	Cattle	Spain	AY159540	[70]
BVDV-1.5	23-15	Cattle	UK	AF298059	[165]
BVDV-1.6 (N)	06z71	Cattle	South Korea	DQ973181	Yang and Kweon, unpubl
BVDV-1.6 (N)	06z127	Cattle	South Korea	DQ973182	Yang and Kweon, unpubl
BVDV-1.6 (N)	KB01	Cattle	South Korea	GQ495676	Oem <i>et al.</i> , unpubl
BVDV-1.6 (M)	S133	Cattle	China	KJ690689	[8]
BVDV-1.6 (N)	Shitara/02/06	Cattle	Japan	LC089876	[91]
BVDV-1.6 (N)	so CP/75	Cattle	Japan	AB042661	[90]
BVDV-1.6 (M)	TY05	Cattle	China	GU120242	[7]
BVDV-1.7 (O)	AQGN96BI5	Cattle	Japan	AB300691	[168]
BVDV-1.7 (M)	BJ09_24	Cattle	China	HQ116550	[8]
BVDV-1.7 (O)	IS25CP/01	Cattle	Japan	AB359931	[28]
BVDV-1.7 (O)	IS26/01ncp	Cattle	Japan	AB359932	[28]
BVDV-1.7 (M)	isolate 9	Bactrian camel	China	JX276546	[31]
BVDV-1.7 (O)	JS10116	Pig	China	JN248734	[32]
BVDV-1.7 (M)	S121	Cattle	China	KF006960	[8]
BVDV-1.7 (M)	S43	Cattle	China	KF006959	[8]
BVDV-1.8	A1-114/AU	Contaminant	Australia	JN967700	[29]
BVDV-1.8 (C)	AQMZ02AI21/2	Cattle	Australia	AB300687	[168]
BVDV-1.8 (C)	Bega	Cattle	Australia	AF049221	Mackintosh <i>et al.</i> , unpubl
BVDV-1.8 (C)	Bega-like	Cattle	Australia	KF896608	Gao <i>et al.</i> , unpubl
BVDV-1.8	CRFK	Contaminant	Japan	D50814	[30]
BVDV-1.8 (C)	isolate 3	Bactrian camel	China	JX276540	[31]
BVDV-1.8 (C)	isolate 4	Bactrian camel	China	JX276541	[31]
BVDV-1.8 (C)	Letuyi	Cattle	China	EU159701	[9]
BVDV-1.8 (C)	Manasi	Cattle	China	EU159702	[9]
BVDV-1.8 (C)	S183	Contaminant	China	KF006968	[8]
BVDV-1.8 (C)	Shitara/01/05	Cattle	Japan	AB359926	[28]
BVDV-1.8 (C)	Shihezi 148	Cattle	China	EU159700	[9]
BVDV-1.9	KM	Cattle	Slovakia	AF298068	[165]
BVDV-1.10 (Q)	11N36	Cattle	China	JX437156	[4]
BVDV-1.10 (Q)	BJ1022	Cattle	China	KF925508	[154]
BVDV-1.10 (Q)	BJ1123	Cattle	China	KF925506	[154]
BVDV-1.10 (Q)	BJ1301	Cattle	China	KF925503	[154]
BVDV-1.10 (Q)	Camel6	Bactrian camel	China	KC695810	[3]
BVDV-1.10 (Q)	FJ1003	Pig	China	JN248728	[32]
BVDV-1.10 (Q)	JL1001	Pig	China	JN248733	[32]
BVDV-1.10 (M)	isolate 6	Bactrian camel	China	JX276543	[31]
BVDV-1.10 (M)	isolate 7	Bactrian camel	China	JX276544	[31]
BVDV-1.10 (Q)	S53	Cattle	China	KF006962	[8]
BVDV-1.10 (Q)	S83	Cattle	China	KF006963	[8]
BVDV-1.10 (Q)	SD0803	Pig	China	JN400273	[107]
BVDV-1.10 (Q)	ZJ1003	Pig	China	JN248744	[32]

## Continued

BVDV-1.10 (Q)	ZJ1005	Pig	China	JN248745	[32]
BVDV-1.11 (E)	S182	Cattle	China	KF006965	[8]
BVDV-1.12.2	11207/98	Cattle	Germany	AJ304390	[154]
BVDV-1.13	KS86-1ncp	Cattle	Japan	AB042713	[90]
BVDV-1.14 (R)	TR70	Cattle	Turkey	MG670547	[15]
BVDV-1.14 (R)	TR75	Cattle	Turkey	MG670549	[15]
BVDV-1.15.1	BJ10_10	Cattle	China	HQ879791	Zhang <i>et al.</i> , unpublsh
BVDV-1.15.1 (M)	BJ1020	Cattle	China	KF925511	[6]
BVDV-1.15.1	BJ11_01	Cattle	China	JN542505	Zhang <i>et al.</i> , unpublsh
BVDV-1.15.1 (M)	BJ1125	Cattle	China	KF925519	[6]
BVDV-1.15.1 (M)	BJ1202	Cattle	China	KF925514	[154]
BVDV-1.15.1 (M)	BJ1302	Cattle	China	KF925520	[154]
BVDV-1.15.1 (M)	BJ1303	Cattle	China	KF925518	[154]
BVDV-1.15.1 (M)	BJ1305	Cattle	China	KF925505	[154]
BVDV-1.15.1 (M)	DG07	Cattle	China	GU120250	[7]
BVDV-1.15.1 (M)	GXBH-EB20	Buffalo	China	KJ578811	[2]
BVDV-1.15.1 (M)	GXBH-EB33	Buffalo	China	KJ578812	[2]
BVDV-1.15.1 (M)	GXBS-LB3	Buffalo	China	KJ578818	[2]
BVDV-1.15.1 (M)	GXHZ-JB24	Buffalo	China	KJ578810	[2]
BVDV-1.15.1 (M)	GXLZ-BB13	Buffalo	China	KJ578817	[2]
BVDV-1.15.1 (M)	GXLZ-BB5	Buffalo	China	KJ578815	[2]
BVDV-1.15.1 (M)	GXLZ-BB6	Buffalo	China	KJ578816	[2]
BVDV-1.15.1 (M)	GXYL-KB22	Buffalo	China	KJ578820	[2]
BVDV-1.15.1 (M)	GXYL-KB53	Buffalo	China	KJ578821	[2]
BVDV-1.15.1 (M)	HB-0244	Cattle	China	KJ578837	[2]
BVDV-1.15.1 (M)	HB-0273	Cattle	China	KJ578831	[2]
BVDV-1.15.1 (M)	HB-0275	Cattle	China	KJ578834	[2]
BVDV-1.15.1 (M)	HB-050030	Cattle	China	KJ578835	[2]
BVDV-1.15.1 (M)	HB-050057	Cattle	China	KJ578841	[2]
BVDV-1.15.1 (M)	HB-090913	Cattle	China	KJ578824	[2]
BVDV-1.15.1 (M)	HZ05	Cattle	China	GU120240	[7]
BVDV-1.15.1 (M)	HZ0601	Cattle	China	GU120244	[7]
BVDV-1.15.1 (M)	HZ0602	Cattle	China	GU120245	[7]
BVDV-1.15.1(M)	isolate 11	Bactrian camel	China	JX276548	[31]
BVDV-1.15.1 (M)	JS-01159	Cattle	China	KJ578843	[2]
BVDV-1.15.1 (M)	JS-03105	Cattle	China	KJ578845	[2]
BVDV-1.15.1 (M)	JS-04198	Cattle	China	KJ578844	[2]
BVDV-1.15.1 (M)	JS-3094	Cattle	China	KJ578842	[2]
BVDV-1.15.1 (M)	LN-1	Cattle	China	KT896495	Zhang, unpublsh
BVDV-1.15.1 (M)	LN317-6	Cattle	China	KJ578795	[2]
BVDV-1.15.1 (M)	LZ05	Cattle	China	GU120241	[7]
BVDV-1.15.1 (M)	NMG311-2	Cattle	China	KJ578867	[2]
BVDV-1.15.1 (M)	NMG311-3	Cattle	China	KJ578865	[2]
BVDV-1.15.1 (M)	NMG312-26	Cattle	China	KJ578864	[2]
BVDV-1.15.1 (M)	NMG312-32	Cattle	China	KJ578863	[2]
BVDV-1.15.1 (M)	NMG313-28	Cattle	China	KJ578870	[2]
BVDV-1.15.1 (M)	NMG313-35	Cattle	China	KJ578869	[2]
BVDV-1.15.1 (M)	NMG313-55	Cattle	China	KJ578868	[2]
BVDV-1.15.1 (M)	NMG314-22	Cattle	China	KJ578858	[2]
BVDV-1.15.1 (M)	NMG314-51	Cattle	China	KJ578860	[2]
BVDV-1.15.1 (M)	NMG314-65	Cattle	China	KJ578872	[2]

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BVDV-1.15.1 (M)	NMG315-1	Cattle	China	KJ578861	[2]
BVDV-1.15.1 (M)	NX0801	Cattle	China	GU120252	[7]
BVDV-1.15.1 (M)	NX0802	Cattle	China	GU120253	[7]
BVDV-1.15.1 (M)	NX0803	Cattle	China	GU120254	[7]
BVDV-1.15.1 (M)	QHQL-216	Yak	China	KJ578881	[2]
BVDV-1.15.1 (M)	QHQL-225	Yak	China	KJ578882	[2]
BVDV-1.15.1 (M)	QHQL-268	Yak	China	KJ578885	[2]
BVDV-1.15.1 (M)	S101	Cattle	China	KJ690686	[8]
BVDV-1.15.1 (M)	S102	Cattle	China	KJ690687	[8]
BVDV-1.15.1 (M)	S12	Cattle	China	KJ690679	[8]
BVDV-1.15.1 (M)	S122	Cattle	China	KJ690688	[8]
BVDV-1.15.1 (M)	S123	Cattle	China	KF006958	[8]
BVDV-1.15.1 (M)	S13	Cattle	China	KJ690680	[8]
BVDV-1.15.1 (M)	S21-China	Cattle	China	KF006961	[8]
BVDV-1.15.1 (M)	S22	Cattle	China	KF006955	[8]
BVDV-1.15.1 (M)	S41	Cattle	China	KJ690681	[8]
BVDV-1.15.1 (M)	S52	Cattle	China	KJ690682	[8]
BVDV-1.15.1 (M)	S62	Cattle	China	KJ690683	[8]
BVDV-1.15.1 (M)	S71	Cattle	China	KF006956	[8]
BVDV-1.15.1 (M)	S73	Cattle	China	KJ690684	[8]
BVDV-1.15.1 (M)	S81	Cattle	China	KJ690685	[8]
BVDV-1.15.1 (M)	SD-15	Cattle	China	KR866116	[10]
BVDV-1.15.1 (M)	SH1051	Pig	China	JN248740	[32]
BVDV-1.15.1 (M)	TJ0801	Cattle	China	GU120255	[7]
BVDV-1.15.1 (M)	W4-1	Pig	China	Not deposited	Zhang, unpublsh
BVDV-1.15.1 (M)	XA08	Cattle	China	GU120257	[7]
BVDV-1.15.1 (M)	XZ-141	Yak	China	KJ578907	[2]
BVDV-1.15.1 (M)	ZM-95	Pig	China	AF526381	[49]
BVDV-1.15.1 (M)	ZD05	Cattle	China	GU120243	[7]
BVDV-1.15.2 (M)	HB-0105	Cattle	China	KJ578830	[2]
BVDV-1.15.2 (M)	HB-050062	Cattle	China	KJ578838	[2]
BVDV-1.15.2 (M)	HB-060111	Cattle	China	KJ578822	[2]
BVDV-1.15.2 (M)	HB-080141	Cattle	China	KJ578823	[2]
BVDV-1.15.2 (M)	HB-080146	Cattle	China	KJ578839	[2]
BVDV-1.15.2 (M)	HB-080147	Cattle	China	KJ578840	[2]
BVDV-1.15.2 (M)	HB-090268	Cattle	China	KJ578832	[2]
BVDV-1.15.2(M)	isolate 10	Bactrian camel	China	JX276547	[31]
BVDV-1.15.2 (M)	JS-03140	Cattle	China	KJ578846	[2]
BVDV-1.15.2 (M)	LN309-9	Cattle	China	KJ578804	[2]
BVDV-1.15.2 (M)	LN314-21	Cattle	China	KJ578802	[2]
BVDV-1.15.2 (M)	NMG313-1	Cattle	China	KJ578871	[2]
BVDV-1.15.2 (M)	NMG314-60	Cattle	China	KJ578859	[2]
BVDV-1.15.2 (M)	QHHY-22	Yak	China	KJ578903	[2]
BVDV-1.15.2 (M)	QHTJ-303887	Yak	China	KJ578899	[2]
BVDV-1.15.2 (M)	S72	Cattle	China	KF006957	[8]
BVDV-1.15.2 (M)	XZ-103	Yak	China	KJ578916	[2]
BVDV-1.15.2 (M)	XZ-109	Yak	China	KJ578917	[2]
BVDV-1.15.2 (M)	XZ-133	Yak	China	KJ578906	[2]
BVDV-1.15.2 (M)	XZ-176	Yak	China	KJ578905	[2]
BVDV-1.15.2 (M)	XZ-24	Yak	China	KJ578918	[2]

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BVDV-1.15.2 (M)	XZ-25	Yak	China	KJ578908	[2]
BVDV-1.15.2 (M)	XZ-48	Yak	China	KJ578909	[2]
BVDV-1.15.2 (M)	XZ-69	Yak	China	KJ578910	[2]
BVDV-1.15.2 (M)	XZ-71	Yak	China	KJ578912	[2]
BVDV-1.15.2 (M)	XZ-84	Yak	China	KJ578913	[2]
BVDV-1.15.2 (M)	XZ-86	Yak	China	KJ578914	[2]
BVDV-1.15.2 (M)	XZ-92	Yak	China	KJ578915	[2]
BVDV-1.16 (L)	TR16	Cattle	Turkey	MG670548	[15]
BVDV-1.16 (L)	TR72	Cattle	Turkey	MG670546	[15]
BVDV-1.16 (L)	TR-2007-A-2368MS	Cattle	Turkey	EU716148	[33]
BVDV-1.16 (L)	TR-2007-Gu-175454-4695	Cattle	Turkey	EU716150	[33]
BVDV-1.17 (F)	J	Cattle	Austria	AF298067	[165]
BVDV-1.18 (P)	BJ0701	Cattle	China	GU120247	[7]
BVDV-1.18 (P)	BJ0702	Cattle	China	GU120248	[7]
BVDV-1.18 (P)	BJ0703	Cattle	China	GU120249	[7]
BVDV-1.18 (M)	isolate 5	Bactrian camel	China	JX276542	[31]
BVDV-1.18 (M)	isolate 8	Bactrian camel	China	JX276545	[31]
BVDV-1.18 (P)	TJ06	Cattle	China	GU120246	[7]
BVDV-1.19 (G)	A	Cattle	Austria	AF298064	[165]
BVDV-1.20.1 (X)	CH-01-08	Cattle	Switzerland	EU180024	[54]
BVDV-1.21.2 (U)	GXBH-EB34	Buffalo	China	KJ578813	[2]
BVDV-1.21.2 (U)	GXBS-LB8	Buffalo	China	KJ578819	[2]
BVDV-1.21.2 (U)	GXCZ-FB7	Buffalo	China	KJ578806	[2]
BVDV-1.21.2 (U)	GXCZ-FB22	Buffalo	China	KJ578807	[2]
BVDV-1.21.2 (U)	GXCZ-FB25	Buffalo	China	KJ578808	[2]
BVDV-1.21.2 (U)	GXHZ-JB11	Buffalo	China	KJ578809	[2]
BVDV-1.21.2 (U)	GXLZ-BB4	Buffalo	China	KJ578814	[2]
BVDV-1.21.2 (U)	HB-090166	Cattle	China	KJ578836	[2]
BVDV-1.21.2 (U)	JS-00108	Cattle	China	KJ578848	[2]
BVDV-1.21.2 (U)	JS-0197	Cattle	China	KJ578853	[2]
BVDV-1.21.2 (U)	JS-02007	Cattle	China	KJ578854	[2]
BVDV-1.21.2 (U)	JS-03148	Cattle	China	KJ578850	[2]
BVDV-1.21.2 (U)	JS-03198	Cattle	China	KJ578851	[2]
BVDV-1.21.2 (U)	JS-04119	Cattle	China	KJ578855	[2]
BVDV-1.21.2 (U)	JS-04138	Cattle	China	KJ578852	[2]
BVDV-1.21.2 (U)	JS-05002	Cattle	China	KJ578857	[2]
BVDV-1.21.2 (U)	JS-99054	Cattle	China	KJ578847	[2]
BVDV-1.21.2 (U)	JS-X02126	Cattle	China	KJ578856	[2]
BVDV-1.21.2 (U)	LN309-5	Cattle	China	KJ578803	[2]
BVDV-1.21.2 (U)	M31182	Yak	China	JQ799141	Sun <i>et al.</i> , unpublish
BVDV-1.21.2 (U)	NMG311-20	Cattle	China	KJ578866	[2]
BVDV-1.21.2 (U)	NMG315-5	Cattle	China	KJ578862	[2]
BVDV-1.21.2 (U)	QHQL-252	Yak	China	KJ578884	[2]
BVDV-1.22 (K)	Rebe	Cattle	Switzerland	AF299317	[152]
BVDV-1.23 (P)	S153	Cattle	China	KF006964	[165]
BVDV-2a1.1	167 237	Sheep	UK	U65055	[164]
BVDV-2a1.1	168 149	Sheep	UK	U65056	[164]
BVDV-2a1.1	173 157	Sheep	UK	U65058	[164]
BVDV-2a1.1	175 375	Sheep	UK	U65059	[164]
BVDV-2a1.1	BSE921	Cattle	Belgium	ALIGN_000012	[104]
BVDV-2a1.1	CPA	Contaminant	Japan	D50812	[30]

## Continued

BVDV-2a1.1	CPAE	Contaminant	Japan	D50813	[30]
BVDV-2a1.1	EBTr	Contaminant	Japan	D50817	[116]
BVDV-2a1.1	HE727	Contaminant	Japan	D31807	[30]
BVDV-2a1.1	Lees	Sheep	UK	U65051	[164]
BVDV-2a1.1	MMR-T	Contaminant	Japan	D26052	[117]
BVDV-2a1.1	MP	Contaminant	Belgium	ALIGN_000012	[104]
BVDV-2a1.1	Parvo	Contaminant	Japan	D26614	[20]
BVDV-2a1.1	Rubella	Contaminant	Japan	D26048	[20]
BVDV-2a1.2	15-103	Cattle	France	AF298055	[165]
BVDV-2a1.2	17583-97	Cattle	USA	AF039176	[158]
BVDV-2a1.2	23025	Cattle	USA	AF039172	[158]
BVDV-2a1.2	37Gr	Cattle	Austria	EU327594	[163]
BVDV-2a1.2	7937	Cattle	USA	AF039175	[158]
BVDV-2a1.2	A4-124/US	Contaminant	USA	JN967705	[29]
BVDV-2a1.2	AZ Spl	Cattle	USA	Not deposited	[147]
BVDV-2a1.2	BSE1239	Cattle	Belgium	ALIGN_000012	[104]
BVDV-2a1.2	BSE341	Cattle	Belgium	ALIGN_000012	[104]
BVDV-2a1.2	CH515	Cattle	Chile	AY671985	[144]
BVDV-2a1.2	CH649	Cattle	Chile	AY671986	[144]
BVDV-2a1.2	CH693	Cattle	Chile	AF356505	[144]
BVDV-2a1.2	CH809	Cattle	Chile	AY671987	[144]
BVDV-2a1.2	E5-160/US	Contaminant	USA	JN967726	[29]
BVDV-2a1.2	IT-1732	Contaminant	Italy	AJ416018	Muscillo, unpubl Li <i>et al.</i> , unpubl
BVDV-2a1.2 (a)	JZ05-1	Cattle	China	GQ888686	[162]
BVDV-2a1.2	Kosice	Cattle	Slovakia	EU360934	[147]
BVDV-2a1.2	MAD Spl	Cattle	USA	Not deposited	[147]
BVDV-2a1.2	MN Fetus	Cattle	USA	Not deposited	[147]
BVDV-2a1.2	NY93	Cattle	USA	AF039173	[158]
BVDV-2a1.2	Q126	Cattle	Canada	L32890	[143]
BVDV-2a1.2 (a)	SD-06	Cattle	China	FJ795044	Zhu <i>et al.</i> , unpubl
BVDV-2a1.2	WG4622	Contaminant	Netherland	ALIGN_000012	[104]
BVDV-2a1.2	WVD829	Cattle	Belgium	ALIGN_000012	[104]
BVDV-2a1.2	V-FLL	Cattle	Japan	AB019687	[44]
BVDV-2a1.3	104/98	Cattle	Germany	AJ304381	[154]
BVDV-2a1.3	4-5174	Cattle	France	AF298063	[165]
BVDV-2a1.3	B52-2	Cattle	Germany	Not deposited	[101]
BVDV-2a1.3	CD87	Cattle	Canada	L32887	[143]
BVDV-2a1.3	i4083	Cattle	Argentina	AF417995	[122]
BVDV-2a1.3	i61380	Cattle	Argentina	AF417986	[122]
BVDV-2a1.3	i628	Cattle	Argentina	AF417985	[122]
BVDV-2a1.3	Munich 1	Cattle	Germany	Not deposited	[101]
BVDV-2a1.3	Munich 2	Cattle	Germany	Not deposited	[101]
BVDV-2a1.4 (a)	11-Mi-97	Cattle	Italy	AJ293603	[130]
BVDV-2a1.4	5521-95	Cattle	USA	AF039174	[158]
BVDV-2a1.4	713-2	Cattle	USA	AF039177	[158]
BVDV-2a1.4	97/730	Cattle	NewZealand	AF026770	Vilcek <i>et al.</i> , unpubl
BVDV-2a1.4	BM01 isolate 11	Sheep	Tunisia	AF462006	Thabti <i>et al.</i> , unpubl
BVDV-2a1.4	D1-152/US	Contaminant	USA	JN967717	[29]
BVDV-2a1.4 (a)	HB-1511	Cattle	China	KX096718	Li and Wu, unpubl
BVDV-2a1.4 (a)	HLJ-10	Contaminant	China	JF714967	[128]
BVDV-2a1.4	XJ-04	Cattle	China	FJ527854	Zhu <i>et al.</i> , unpubl

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BVDV-2a1.4	SH-28	Pig	China	HQ258810	[155]
BVDV-2a1.4	UVR420	Cattle	Belgium	ALIGN_000012	[104]
BVDV-2a1.5	BD-78	Sheep	USA	U18330	[153]
BVDV-2a1.5	C413	Sheep	USA	AF002227	Chen & Berry, unpublsh
BVDV-2a1.5	K1-2/CA	Contaminant	Canada	JN967735	[29]
BVDV-2a1.6	890	Cattle	Canada	L32886	[143]
BVDV-2a2.1.1	AF112	Cattle	Germany	Not deposited	[101]
BVDV-2a2.1.1	B45-5	Cattle	Germany	Not deposited	[101]
BVDV-2a2.1.1	B50-5	Cattle	Germany	Not deposited	[101]
BVDV-2a2.1.1	B5-4	Cattle	Germany	Not deposited	[101]
BVDV-2a2.1.1	BA/35/02	Cattle	Italy	AM749820	[110]
BVDV-2a2.1.1	BS-95-II	Cattle	Italy	AJ288903	[130]
BVDV-2a2.1.1	E1-198/CA	Contaminant	Canada	JN967719	[29]
BVDV-2a2.1.1	Giessen-1	Cattle	Germany	AF104030	[61]
BVDV-2a2.1.1	LO/04/04	Cattle	Italy	AM749821	[110]
BVDV-2a2.1.1	LO/08/04	Cattle	Italy	AM749822	[110]
BVDV-2a2.1.1	LO/10/04	Cattle	Italy	AM749823	[110]
BVDV-2a2.1.1	LO/13/04	Cattle	Italy	AM749824	[110]
BVDV-2a2.1.1	MS-1	Cattle	Japan	AB019688	[44]
BVDV-2a2.1.1	Munich 3	Cattle	Germany	Not deposited	[9]
BVDV-2a2.1.1	OY89	Cattle	Japan	AB003621	[139]
BVDV-2a2.1.1 (a)	PT4-02	Cattle	Portugal	AY944291	[99]
BVDV-2a2.1.1	SW90	Cattle	Japan	AB003622	[90]
BVDV-2a2.1.1	SY-89	Cattle	Japan	AB019689	[44]
BVDV-2a2.1.1	TC Shinozaki	Cattle	Japan	AB04267	[90]
BVDV-2a2.1.1 (c)	Bov/Ita/187.15-38	Cattle	Italy	KX350078	[105]
BVDV-2a2.1.1 (c)	Bov/Ita/232.03	Cattle	Italy	KX350067	[105]
BVDV-2a2.1.1 (c)	Cp/Ita/124.15-20	Goat	Italy	KX350077	[105]
BVDV-2a2.1.1 (c)	Cp/Ita/124.15-23	Goat	Italy	KX350076	[105]
BVDV-2a2.1.1 (c)	Cp/Ita/190.15-36	Goat	Italy	KX350069	[105]
BVDV-2a2.1.1 (c)	Cp/Ita/191.15-21	Goat	Italy	KX350075	[105]
BVDV-2a2.1.1 (c)	Ov/Ita/182.15-33	Sheep	Italy	KX350068	[105]
BVDV-2a2.1.1 (c)	Ov/Ita/188.15-18	Sheep	Italy	KX350074	[105]
BVDV-2a2.1.1 (c)	Ov/Ita/189.15-97	Sheep	Italy	KX350072	[105]
BVDV-2a2.1.1 (c)	Ov/Ita/189.15-69	Sheep	Italy	KX350073	[105]
BVDV-2a2.1.1 (c)	Ov/Ita/191.15-16	Sheep	Italy	KX350071	[105]
BVDV-2a2.1.1 (c)	Ov/Ita/192.15	Sheep	Italy	KX350070	[105]
BVDV-2a2.1.2	17011-96	Cattle	USA	AF039179	[158]
BVDV-2a2.2	B77-5	Cattle	Germany	Not deposited	[101]
BVDV-2a3 (c)	301/09	Cattle	Italy	KY040413	[88]
BVDV-2b1.1	F1-5/BR	Contaminant	Brazil	JN967743	[29]
BVDV-2b1.1	VS-63	Cattle	Brazil	AF410789	[109]
BVDV-2b1.2	VS-123.4	Cattle	Brazil	AF410790	[109]
BVDV-2b1.3	34b	Cattle	Argentina	AF244952	[121]
BVDV-2b1.3	ncp7	Cattle	Argentina	Not deposited	[121]
BVDV-2b1.4 (b)	4p	Human	Brazil	MG436781	Tanuri <i>et al.</i> , unpublsh
BVDV-2b1.4 (b)	LV56-10/13	Cattle	Brazil	KM007124	[69]
BVDV-2b1.4 (b)	LV/Patol02/09	Cattle	Brazil	KP715137	Silveira <i>et al.</i> , unpublsh
BVDV-2b1.5	Giessen 6	Cattle	Germany	AY379547	Kuehne <i>et al.</i> , unpublsh
BVDV-2b1.5	VM96	Cattle	Brazil	KP715141	Silveira <i>et al.</i> , unpublsh
BVDV-2b1.5	VS-260	Cattle	Brazil	AF410788	[109]

## Continued

BVDV-2b1.5	LV-96	Cattle	Brazil	AF410787	[109]
BVDV-2b2	B5-135/MX	Contaminant	Mexico	JN967713	[29]
BVDV-2b3	Soldan	Cattle	Brazil	U94914	Canal <i>et al.</i> , unpublsh
BVDV-2b4 (b)	SD-1301	Cattle	China	KJ000672	[5]
BVDV-2b4 (b)	S143	Contaminant	China	KF006972	[8]
BVDV-2b4 (b)	S172	Contaminant	China	KF006973	[8]
BVDV-2b4 (b)	S51	Contaminant	China	Not deposited	[8]
BVDV-2c	098	Sheep	Tunisia	AF462004	Thabti <i>et al.</i> , unpublsh
BVDV-2c	119	Sheep	Tunisia	AF462003	Thabti <i>et al.</i> , unpublsh
BVDV-2c	63	Sheep	Tunisia	AF462005	Thabti <i>et al.</i> , unpublsh
BVDV-2d	A3-118/US	Contaminant	USA	JN967703	[29]
BVDV-2e	i33283	Cattle	Argentina	AF417996	[122]
BVDV-2f1	354	Contaminant	Argentina	AF244959	[121]
BVDV-2f2 (b)	PT2-01	Cattle	Portugal	AY944277	[99]
BVDV-2f2 (b)	PT7-02	Cattle	Portugal	AY944297	[99]
BVDV-2f2	Ptn3	Cattle	Portugal	EU034173	Mota, unpublsh
BVDV-2f2	Ptn4	Cattle	Portugal	EU034174	Mota, unpublsh
BVDV-2f2	Ptn8	Cattle	Portugal	EU034175	Mota, unpublsh
BVDV-2nd	59386	Sheep	UK	U17146	[100]
BVDV-2nd	SCP	Contaminant	UK	U17148	[100]
BVDV-3.1.1.1	A2/BR	Contaminant	Brazil	JN967701	[29]
BVDV-3.1.1.1	Au/A55110-1162/09	Contaminant	Australia	FR873797	[167]
BVDV-3.1.1.1	Au/9SB007/09	Contaminant	Australia	FR873798	[167]
BVDV-3.1.1.1	B1/AU	Contaminant	Australia	JN967707	[29]
BVDV-3.1.1.1	B2-42/AU	Contaminant	Australia	JN967709	[29]
BVDV-3.1.1.1	B3/AU	Contaminant	Australia	JN967710	[29]
BVDV-3.1.1.1	B4-3/CA	Contaminant	Canada	JN967711	[29]
BVDV-3.1.1.1	B5-3/MX	Contaminant	Mexico	JN967747	[29]
BVDV-3.1.1.1	B6/US	Contaminant	USA	JN967714	[29]
BVDV-3.1.1.1	D32/00_HoBi/Brazil/200/2002	Cattle	Brazil	EF683557	[98]
BVDV-3.1.1.1	D32/00_HoBi	Cattle	Brazil	AY489116	[84]
BVDV-3.1.1.1	E4/SA	Contaminant	South American	JN967724	[29]
BVDV-3.1.1.1	F1-29/BR	Contaminant	Brazil	JN967727	[29]
BVDV-3.1.1.1	G1-35/AU	Contaminant	Australia	JN967728	[29]
BVDV-3.1.1.1	G2/BR	Contaminant	Brazil	JN967729	[29]
BVDV-3.1.1.1	H1-50/AU	Contaminant	Australia	JN967731	[29]
BVDV-3.1.1.1	H3-3/US	Contaminant	USA	JN967748	[29]
BVDV-3.1.1.1	Hobi/Brazil/315/2004	Cattle	Brazil	EF683558	[98]
BVDV-3.1.1.1	Italy-1/10-1	Cattle	Italy	HQ231763	[85]
BVDV-3.1.1.1	Italy-280/11-A	Cattle	Italy	JN703311	[86]
BVDV-3.1.1.1	Italy-83/10-cp	Cattle	Italy	JQ612705	[87]
BVDV-3.1.1.1	Italy-83/10-ncp	Cattle	Italy	JQ612704	[87]
BVDV-3.1.1.1	IZSPLV_To	Contaminant	Italy	HM151361	[142]
BVDV-3.1.1.1	LV02/12	Cattle	Brazil	KC465389	Weber <i>et al.</i> , unpublsh
BVDV-3.1.1.1	LV03/12	Cattle	Brazil	KC465390	Weber <i>et al.</i> , unpublsh
BVDV-3.1.1.1	LV04/12	Cattle	Brazil	KC465391	Weber <i>et al.</i> , unpublsh
BVDV-3.1.1.1	LPV-WR/BR11	Cattle	Brazil	KC544256	Cruz <i>et al.</i> , unpublsh
BVDV-3.1.1.1	PB22487	Cattle	Brazil	KY762287	[136]
BVDV-3.1.1.1	SV478/07	Cattle	Brazil	KY767958	[136]
BVDV-3.1.1.2	JS12/01	Contaminant	China	JX469119	[35]
BVDV-3.1.1.3	Th/04_KhonKaen	Cattle	Thailand	DQ897641	[36]



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BVDV-3.1.2	HN1524	Sheep	China	KU053493	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1542	Sheep	China	KU053496	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1559	Sheep	China	KU053497	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1519	Sheep	China	KU053492	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1537	Sheep	China	KU053495	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1528	Sheep	China	KU053494	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1518	Sheep	China	KU053491	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1513	Goat	China	KU053490	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1507	Goat	China	KU563155	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1568	Sheep	China	KU053499	Shi <i>et al.</i> , unpubl
BVDV-3.1.2	HN1564	Sheep	China	KU053498	Shi <i>et al.</i> , unpubl
BVDV-3.1.3	IndABI16023/12	Zebu	India	KM201316	[13]
BVDV-3.1.3	IndABI15385/12	Cattle	India	KM201313	[13]
BVDV-3.1.3	IndABI15987/12	Zebu	India	KM201314	[13]
BVDV-3.1.3	IndABI16020/12	Zebu	India	KM201315	[13]
BVDV-3.1.4	BGDZS1	Cattle	Bangladesh	KF204448	[12]
BVDV-3.2	IndBHA5296/12	Zebu	India	KM201299	[13]
BVDV-3.2	IndBHA5309/12	Zebu	India	KM201300	[13]
BVDV-3.2	IndBHA5426/12	Zebu	India	KM201301	[13]
BVDV-3.2	IndBHA5500/12	Zebu	India	KM201302	[13]
BVDV-3.2	IndBHA6060/12	Zebu	India	KM201303	[13]
BVDV-3.2	IndBHA6229/12	Zebu	India	KM201304	[13]
BVDV-3.2	IndBHA6332/12	Zebu	India	KM201305	[13]
BVDV-3.2	IndBHA6603/12	Zebu	India	KM201306	[13]
BVDV-3.2	IndBHA6604/12	Zebu	India	KM201307	[13]
BVDV-3.2	IndBHA6610/12	Zebu	India	KM201308	[13]
BVDV-3.2	IndMDV18963/12	Zebu	India	KM201318	[13]
BVDV-3.2	IndNAR0115/12	Zebu	India	KM201311	[13]
BVDV-3.2	IndNAR0116/12	Zebu	India	KM201312	[13]
BVDV-3.3	BGD/ZS5	Cattle	Bangladesh	KF204450	[12]
BVDV-3.4	BGD/ZS3	Cattle	Bangladesh	KF204449	[12]
BDV-a1 (1a)	A841/1	Sheep	UK	U65026	[164]
BDV-a2 (1b)	8320-22NZ	Sheep	New Zealand	U65063	[164]
BDV-a2 (1b)	8320-31NZ	Sheep	New Zealand	U65064	[164]
BDV-a2 (1b)	Ch1Es	Contaminant	Japan	D50816	[30]
BDV-a2	FNK2012-1	Pig	Japan	AB897785	[137]
BDV-a2 (1)	FNK2012-2	Pig	Japan	AB846840	[125]
BDV-a2 (1)	FNK2012-3	Pig	Japan	AB846841	[125]
BDV-a2	FNK2012-4	Pig	Japan	AB846842	[125]
BDV-a2	Lyon2	Black goat	South Korea	DQ350165	Kim <i>et al.</i> , unpubl
BDV-a2 (1b)	X818	Sheep	Australia	AF037405	[100]
BDV-a3 (1b)	BD31	Sheep	USA	U70263	[146]
BDV-a4 (1)	LA/5909/09	Sheep	Italy	LM999986	[111]
BDV-b (2)	Rentier Rudolph	Reindeer	Germany	AB122086	[67]
BDV-c1.1 SW	CH-BD3	Sheep	Switzerland	JQ994199	Stalder <i>et al.</i> , unpubl
BDV-c1.2 (8)	Italy-103761	Goat	Italy	KT072634	[141]
BDV-c2	92-F-7119	Sheep	France	EF693994	[108]
BDV-c3 (8)	Italy-58987	Alpine Chamois	Italy	KX573913	[102]
BDV-d1 (3)	AH12-01	Goat	China	JQ946320	[37]
BDV-d1	AH12-02	Goat	China	JX437132	[37]
BDV-d1	AHHX15	Sheep	China	KT327870	Li <i>et al.</i> , unpubl

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BDV-d2	(3)	297	Sheep	Slovakia	KC484999	[38]
BDV-e1	(4)	C27	Sheep	Spain	DQ275623	[161]
BDV-e2	(4b)	ZA1-1115	Sheep	Spain	DQ361070	[160]
BDV-f1	(5)	AV	Sheep	France	EF693984	[108]
BDV-f2	(4c)	2112/99	Sheep	Spain	AY159513	[70]
BDV-f3	(4b)	Rocco	Sheep	Spain	DQ361067	[160]
BDV-f4	(4)	Chamois-1 (H2121)	Pyrenean chamois	Andorra	AY738080	[64]
BDV-g1		91-F-6732	Sheep	France	EF988633	[108]
BDV-g2	TU	37A	Contaminant	Tunisia	AF461999	[156]
BDV-h	(3)	JS12/04	Goat	China	KC537789	[37]
BDV-h		JSL12-01	Sheep	China	KC963426	[129]
BDV-h		JSYZ15	Sheep	China	KT327869	Li <i>et al.</i> , unpublsh
BDV-i1		TR-13	Sheep	Turkey	JF489888	[39]
BDV-i2		TR-14	Sheep	Turkey	JF489889	[39]
BDV-j1	(3)	Ind 830-09	Sheep	India	KT934377	[40]
BDV-j2	(3)	CH-BD1	Sheep	Switzerland	JQ994201	Stalder, <i>et al.</i> , unpublsh
BDV-j3	(3)	Gifhorn	Pig	Germany	EU636997	Schirmeier <i>et al.</i> , unpublsh
<i>Pestivirus I</i>		BDV/Aydin/04-TR	Sheep	Turkey	AM418427	[57]
<i>Pestivirus I</i>		BDV/Burdur/05-TR	Sheep	Turkey	AM418428	[57]
CSFV-a1		1 India	Cattle	India	MG859286	[11]
CSFV-a1		10 India	Cattle	India	MK105823	[11]
CSFV-a1		11 India	Cattle	India	MG859287	[11]
CSFV-a1		13 India	Cattle	India	MK105824	[11]
CSFV-a1		2 India	Cattle	India	MK105825	[11]
CSFV-a1		39	Pig	China	AF407339	Wu <i>et al.</i> , unpublsh
CSFV-a1		3 India	Cattle	India	MK105826	[11]
CSFV-a1		4 India	Cattle	India	MK105820	[11]
CSFV-a1		5 India	Cattle	India	MK105821	[11]
CSFV-a1		5NCR/CSF/MZ/AIZ/348	Pig	India	JX975460	Rajkhowa <i>et al.</i> , unpublsh
CSFV-a1		5NCR/CSF/MZ/AIZ/352	Pig	India	JX975461	Rajkhowa <i>et al.</i> , unpublsh
CSFV-a1		6 India	Cattle	India	MK105822	[11]
CSFV-a1		7 India	Cattle	India	MK109913	[11]
CSFV-a1		8 India	Cattle	India	MG813566	[11]
CSFV-a1		9 India	Cattle	India	MK105827	[11]
CSFV-a1	(1.1)	Alfort 187	Pig	France	X87939	[149]
CSFV-a1		Alfort A19	Pig	France	U90951	Smondack <i>et al.</i> , unpublsh
CSFV-a1		Aizawl-09	Pig	India	HM449066	Rajkhowa <i>et al.</i> , unpublsh
CSFV-a1		Bangalore Ind-163/07	Pig	India	EU446419	Patil <i>et al.</i> , unpublsh
CSFV-a1	(1.1)	Brescia	Pig	Italy	M31768	[134]
CSFV-a1	(1.2)	BRESCIAX	Pig	Italy	AY578687	[148]
CSFV-a1		BV-P	Pig	Hungary	DQ314582	Farsang <i>et al.</i> , unpublsh
CSFV-a1		C strain	Vaccine	China	Z46258	[135]
CSFV-a1	(1.1)	CAP	Pig	Switzerland	X96550	Tratschin <i>et al.</i> , unpublsh
CSFV-a1		cF114	Pig	China	AF333000	Mingxiao <i>et al.</i> , unpublsh
CSFV-a1		CSF/MZ/KOL/73	Pig	India	JX094153	Rajkhowa, unpublsh
CSFV-a1		CSF/MZ/SAI/76	Pig	India	JX094154	Rajkhowa, unpublsh
CSFV-a1	(1.1)	CSFV/1.1/dp/CSF0382/Koslov	Pig	Czech Republic	HM237795	[126]
CSFV-a1	(2.3)	CSFV/2.3/wb/0608/2005/Euskirchen	Wild boar	Germany	GU233732	[126]
CSFV-a1	(2.3)	CSFV/2.3/wb/CSF1046/2009/Hennef	Wild boar	Germany	GU233733	[126]
CSFV-a1	(2.3)	CSFV/2.3/wb/CSF1045/2009/Roesrath	Pig	Germany	LT593749	Hoepfer, unpublsh
CSFV-a1	(1.1)	CSFV212L-13	Pig	India	KY860615	Tomar <i>et al.</i> , unpublsh

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CSFV-a1	(1.1)	CSFV-GZ-2009	Pig	China	HQ380231	[150]
CSFV-a1	(1.1)	CSFV/IVRI/VB-131	Pig	India	KM262189	[124]
CSFV-a1	(1.1)	CSFV-PK15C-NG79-11	Contaminant	India	KC503764	[157]
CSFV-a1		Eystrup	Pig	Germany	AF326963	[133]
CSFV-a1		GPE (-)	Vaccine	Japan	AB019152	[43]
CSFV-a1	(1.1)	HCLV	Vaccine	India	AF091507	Wang <i>et al.</i> , unpublsh
CSFV-a1	(1.1)	HCVCAD22/14	Contaminant	India	U606028	Desai <i>et al.</i> , unpublsh
CSFV-a1		Hokkaido/66	Pig	Japan	AB019154	[43]
CSFV-a1		Ibaraki/66	Pig	Japan	AB019156	[43]
CSFV-a1		Ibaraki/81-115	Pig	Japan	AB019158	[43]
CSFV-a1		Ibaraki/81-20	Pig	Japan	AB019160	[43]
CSFV-a1		Ibaraki/81-38	Pig	Japan	AB019162	[43]
CSFV-a1		Ibaraki/81-40	Pig	Japan	AB019164	[43]
CSFV-a1		Ind-173/08	Pig	India	FJ183444	[140]
CSFV-a1		Ind-174/08	Pig	India	FJ183445	[140]
CSFV-a1		Ind-175/08	Pig	India	FJ183446	[140]
CSFV-a1		Ind-176/08	Pig	India	FJ183447	[140]
CSFV-a1		Ind-239/08	Pig	India	FJ183449	[140]
CSFV-a1		Ind-243/08	Pig	India	FJ183452	[140]
CSFV-a1		Ind-272/08	Pig	India	FJ183456	[140]
CSFV-a1	(1.1)	JL1(06)	Pig	China	EU497410	Qiu <i>et al.</i> , unpublsh
CSFV-a1		KC	Vaccine	Russia	AF099102	[114]
CSFV-a1		LK-VNIVViM	Vaccine	Russia	KM522833	[169]
CSFV-a1	(1.1)	LOM	Pig	Japan	AB019655	[44]
CSFV-a1		Miyazaki/81	Pig	Japan	AB019168	[43]
CSFV-a1		Nakamura/66	Pig	Japan	AB019170	[43]
CSFV-a1	(1.1)	NFP/AS-1	Pig	India	KC617749	Roychoudhury <i>et al.</i> , unpublsh
CSFV-a1	(1.1)	NFP/ML-2	Pig	India	KC617761	Roychoudhury <i>et al.</i> , unpublsh
CSFV-a1	(1.1)	NFP/ML-4	Pig	India	KC617750	Roychoudhury <i>et al.</i> , unpublsh
CSFV-a1		Rovac	Vaccine	USA	KJ873238	[169]
CSFV-a1	(1.2)	RUCSFPLUM	Vaccine	USA	AY578688	[148]
CSFV-a1		Shimen	Pig	China	AF092448	Huang <i>et al.</i> , unpublsh
CSFV-a1		SWH	Pig	China	DQ127910	[127]
CSFV-a1		Vac A	Pig	USA	L42435	[151]
CSFV-a1		Yamanashi/69	Pig	Japan	AB019182	[43]
CSFV-a2		17-93	Pig	Poland	L42413	[151]
CSFV-a2	(2.3)	Alfort/Tübingen	Pig	France	J04358	[132]
CSFV-a2		Chiba-80	Pig	Japan	AB019659	[44]
CSFV-a2	(2.3)	CSFV/2.3/dp/CSF0821/HR/Novska	Pig	Croatia	HQ148061	Leifer <i>et al.</i> , unpublsh
CSFV-a2	(2.3)	CSFV/2.3/dp/CSF857/Borken	Pig	Germany	GU233731	[126]
CSFV-a2	(2.3)	CSFV/2.3/dp/CSF864/BG/Jambul	Pig	Bulgaria	HQ148062	Leifer <i>et al.</i> , unpublsh
CSFV-a2	(2.3)	CSFV/2.3/wb/XXX0609/Uelzen	Wild boar	Germany	GU324242	[126]
CSFV-a2	(2.3)	CSFV/2.3/SRB/1264/2005	Pig	Serbia	KY849593	Petrovic <i>et al.</i> , unpublsh
CSFV-a2	(2.3)	CSFV/2.3/SRB/6168/2006	Pig	Serbia	KY849594	Petrovic <i>et al.</i> , unpublsh
CSFV-a2		CSF/wb/FR57/2004/0964-03	Pig	Germany	LT158404	Hoeper, unpublsh
CSFV-a2		CSF/wb/FR67/2003/0647-19	Pig	Germany	LT158502	Hoeper, unpublsh
CSFV-a2		CSF/wb/FR67/2005/0018-06	Pig	Germany	LT158405	Hoeper, unpublsh
CSFV-a2		CSF/wb/FR67/2005/0125-05	Pig	Germany	LT158406	Hoeper, unpublsh
CSFV-a2		CSF/wb/FR67/2005/0238-02	Pig	Germany	LT158407	Hoeper, unpublsh
CSFV-a2		CSF/wb/FR67/2006/0199-01	Pig	Germany	LT158409	Hoeper, unpublsh
CSFV-a2		CSF/wb/FR67/2006/060003-06	Pig	Germany	LT158408	Hoeper, unpublsh

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CSFV-a2	CSF/wb/FR67/2007/0192-01	Pig	Germany	LT158410	Hoeper, unpublsh
CSFV-a2 (2.1g)	GD19/2011	Pig	China	KU504339	[113]
CSFV-a2	HEN03	Cattle	China	KC176778	Zhang <i>et al.</i> , unpublsh
CSFV-a2 (2.1)	HNLY-2011	Pig	China	JX262391	[120]
CSFV-a2 (2.1)	HNSD-2012	Pig	China	JX218094	[120]
CSFV-a2	Osaka/51	Pig	Japan	AB019174	[43]
CSFV-a2	Osaka/71	Pig	Japan	AB019176	[43]
CSFV-a2	Pader	Pig	Germany	AY072924	[159]
CSFV-a2	Paderborn	Pig	Germany	GQ902941	[145]
CSFV-a2	S112	Contaminant	China	MK118725	[8]
CSFV-a2	S173	Contaminant	China	KF006975	[8]
CSFV-a2	Shizuoka/73	Pig	Japan	AB019180	[43]
CSFV-a2 (2.1)	SKCDK	Pig	China	GQ923951	Li <i>et al.</i> , unpublsh
CSFV-a2	Sp01	Pig	Spain	FJ265020	Mena <i>et al.</i> , unpublsh
CSFV-a2	Switzerland 1/93	Pig	Switzerland	AF045068	[119]
CSFV-a2	Switzerland 2/93'	Pig	Switzerland	AF045069	[119]
CSFV-a2	Switzerland 3/93/1'	Pig	Switzerland	AF045070	[119]
CSFV-a2	Switzerland 3/93/2'	Pig	Switzerland	AF045071	[119]
CSFV-a2	Switzerland 4/93'	Pig	Switzerland	AF045072	[119]
CSFV-a2	Venhorst	Pig	Netherlands	AF084049	[166]
CSFV-a2	VRI4762	Pig	Malaysia	L42437	[151]
CSFV-a2 (2.3)	Wingene	Pig	Belgium	JQ595295	Haegeman <i>et al.</i> , unpublsh
CSFV-a3	Saitama/81	Pig	Japan	AB019178	[43]
CSFV-a4 (2.2)	179/MIB/2014	Pig	India	KR350485	[42]
CSFV-a4 (2.2)	181/MIB/2014	Pig	India	KR350486	[42]
CSFV-a4 (2.2)	211/MIB/2014	Wild boar	India	KR350487	Ravishankar <i>et al.</i> , unpublsh
CSFV-a4 (2.2)	23/MIB/2014	Pig	India	KR149284	[42]
CSFV-a4 (2.2)	322/MIB/2013	Pig	India	KR350488	[42]
CSFV-a4 (2.2)	99/MIB/2014	Pig	India	KR350483	[42]
CSFV-a4 (2)	CSFV-UP-BR-757-09	Pig	India	KC533785	Tomar <i>et al.</i> , unpublsh
CSFV-a4	Parambi	Wild boar	India	KT239105	Chandramohan <i>et al.</i> , unpublsh
CSFV-a5	IND/AS/GHY/G4	Pig	India	KM362426	[53]
CSFV-a6	Fukuoka/72	Pig	Japan	AB019150	[43]
CSFV-a6	Honduras	Pig	Honduras	L42426	[151]
CSFV-b	5440/99	Sheep	Spain	AY159514	[70]
CSFV-c (3)	94.4/IL/94/TWN	Pig	Taiwan	AY646427	[41]
CSFV-c (3.4)	Kanagawa/74	Pig	Japan	AB019166	[43]
CSFV-c	Okinawa/86	Pig	Japan	AB019172	[43]
CSFV-c	Okinawa-86-2	Pig	Japan	AB019663	[44]
CSFV-c (3)	P97	Pig	Taiwan	L49347	Liu, unpublsh
CSFV-d	S171	Contaminant	China	KF006974	[8]
CSFV nd (2.2)	Sch180	Pig	Germany	JQ411560	[80]
CSFV nd (1.3)	VRI 4167	Pig	Malaysia	JQ411570	[80]

Alpine chamois (*Rupicapra rupicapra*); Bactrian camel (*Camelus bactrianus*); Black Goat (*Capra aegagrus hircus*); Buffalo (*Bubalus bubalis*); Cattle (*Bos taurus*); Goat (*Capra hircus*); Human (*Homo sapiens*); Pig (*Sus scrofa domestica*); Pyrenean chamois (*Rupicapra pyrenaica*); Reindeer (*Rangifer tarandus*); Serow (*Capricornis crispus*); Sheep (*Ovis aries*); Wild boar (*Sus scrofa*); Yak (*Bos grunniens*), Zebu (*Bos indicus*).

sequence was identified from a human pathological sample in Brazil. Seven sequences were CSFV vaccinal strains. Eighty-three strains were contaminants of biological products, mainly detected in fetal bovine serum (FBS) samples for laboratory use, but also cell lines, interferon for human use, vaccines for veterinary

and human use. Most of the samples were from Japan (n 37). Other contaminants were reported from China, India, Australia, New Zealand, United Kingdom, Italy, Belgium, Netherland, Tunisia, USA, Canada, Mexico, Brazil and Argentina. Sequences were compared to previously evaluated strain, reaching a total number of 1426 observations obtained with PNS procedure (Table 2). Qualitative and quantitative evaluation of genomic sequence divergence, in terms of palindromic nucleotide base pairings variations, has been applied for taxonomical segregation, through the evaluation of relevant secondary structure regions in the 5'-UTR of the viral RNA, the three variable regions, V1, V2 and V3 genomic sequences (Table 3), according to the genotyping based on the PNS method [17] [18]. As described by Deng and Brock [19] and Harasawa [20], the 5'-UTR is characterized by a series of secondary sequence stem-loop structures which are divided into 4 domains: A, B, C and D. The most significant is Domain D which corresponds to the IRES and it covers two thirds of the 5'-UTR sequence from nucleotides 139 to 361 and it is conserved among NADL, Osloss, SD-1, Alfort and Brescia *Pestivirus* reference strains [19]. The predicted Domain D complex palindromic stem-loop structures D2, D3 and D4 [19], or as recently renamed as IRES domains IIIb, IIIId1 and IIIId2 [21], correspond to PNS V1, V2 and V3 variable loci, respectively. Within the genus, the palindromic structures identifiable in linear sequences were generally characterized by the absence of nucleotides in position 22 of V1 and V2 locus composed by a constant number of 23 nucleotides.

**Table 2.** Summary of *Pestivirus* strains (n = 1426) evaluated according to the Palindromic nucleotide substitution (PNS) method at the 5' untranslated region of RNA. \*Tentative species.

Species	Number of strains	Host	Geographical origin
BVDV-1	887	Cattle, Alpaca, Alpine chamois, Bactrian camel, Buffalo, Contaminant, Deer, Goat, Human, Mousedeer, Pig, Roe deer, Serow, Sheep, Yak, Zebu.	Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Colombia, Denmark, Dominican Republic, Egypt, France, Germany, India, Ireland, Italy, Japan, Mexico, New Zealand, Poland, Portugal, Slovakia, South Africa, South Korea, Spain, Sweden, Switzerland, Tunisia, Turkey, UK, USA
BVDV-2	130	Cattle, Contaminant, Human, Sheep.	Argentina, Austria, Belgium, Brazil, Canada, Chile, China, France, Germany, Italy, Japan, Mexico, Netherland, New Zealand, Portugal, Slovakia, Spain, Tunisia, UK, USA
BVDV-3	62	Cattle, Buffalo, Contaminant, Goat, Sheep, Zebu.	Australia, Bangladesh, Brazil, Canada, China, India, Italy, Mexico, Thailand, USA
BDV	210	Sheep, Alpine chamois, Black goat, Cattle, Goat, Pig, Pyrenean chamois, Reindeer, Wisent.	Andorra, Australia, Austria, China, France, Germany, Italy, Japan, New Zealand, Slovakia, Spain, South Korea, Switzerland, Tunisia, Turkey, UK, USA
BDV-2*	5	Sheep, Goat.	Italy
<i>Pestivirus</i> I	2	Sheep.	Turkey
CSFV	126	Pig, Cattle, Contaminant, Sheep.	China, France, Germany, Honduras, India, Italy, Japan, Malaysia, Netherlands, Poland, Russia, Spain, Switzerland, Taiwan, USA
Pronghorn	1	Pronghorn.	USA
Giraffe	2	Cattle, Giraffe.	Kenya
Bungowannah	1	Pig.	Australia

**Table 3.** Palindromic structures V1, V2 and V3 variable loci positions in linear sequences of *Pestivirus* BVDV-1, BVDV-2, BVDV-3, BDV and CSFV species reference strains for the evaluation according to secondary structure characteristics at the RNA 5'-UTR (PNS method).

Reference strains	Variable loci			Reference
	V1	V2	V3	
BVDV-1 Osloss	197 - 235	267 - 289	293 - 311	[143]
BVDV-1 NADL	198 - 236	268 - 290	294 - 313	[103]
BVDV-2 890	75 - 116	148 - 170	174 - 191	[106]
BVDV-3 Hobi/Brazil/200/2002	37 - 76	106 - 128	134 - 152	[98]
BDV X818	186 - 224	256 - 278	282 - 297	[100]
CSFV Alfort/Tübingen	187 - 225	257 - 279	283 - 297	[132]

The variation of loops in V1 and V3 loci determined difference in size of palindromes. Secondary structures were obtained for the entire 5'-UTR sequence of each strain. Palindromic sequences corresponding to the IRES three variable loci were identified in the predicted secondary structure and considered out of the rest of the nucleotide sequence. Nucleotide sequence secondary structures were predicted according to the algorithm of Zuker and Stiegler [22] using the Genetyx-Mac version 14 program package (Software Development Co., Ltd., Tokyo, Japan). The minimum free energy was calculated by the method of Freier *et al.* [23]. The PNS software version 2.0 [24], prepared for the application on the genotyping procedures with the keys for *Pestivirus* identification of genomic sequences, using the C# programming language, was also applied for the construction of secondary structure sequence alignment, in order to compute genetic distance among strains. Segregation of *Pestivirus* species strains into genotypes and relatedness among genotypes within the species was evaluated according to changes in nucleotide base pairs at the level of the secondary palindromic structure of the three variable loci. Genotypes have been defined applying a nomenclature reflecting the level of divergence within the species, and ranked according to increasing divergence expressed in number of divergent base pair (bp), with reference to most common observed sequence combinations in the genus. Genotype roots, base-pair (bp) combinations at the level of low-variable positions (LVP), also defined determinative LVP [14] [17], were identified to support genetic clustering of strain sequences and characterize species genotypes, varying for each species (LVP V1/14, V2/7 and V3/4 for BVDV-1; V1/9, V1/12, V1/16, V1/17 and V1/18 for BVDV-2 or V1/9, V2/1, V2/5 and V3/8 for BDV). Among genotypes, homology was evaluated in terms of shared base pairs in the three variable loci. Cross comparison between types within the genus has been evaluated by computing the divergence percentage, identifying strains showing multiple relation (sequences sharing base pairings specific to different genomic groups, and scoring low divergence values) or borderlines (sequences showing qualitative similarities with a genomic group, but

with high divergence values, candidates for reallocation as separate groups in the genus), and indicating divergence within groups and among groups quantifying the heterogeneity of a genotype and the genetic distance between groups. Phylogenetic trees based on the 5'-UTR were constructed following sequence alignment of representative species strains with Clustal X [25] by using the neighbour-joining method [26] and visualized with Newick tree format option (Mega version 7.0.26) [27].

### 3. Results

The observation made on the nucleotide sequences of the three variable loci at the level of the 5'-UTR genomic region of *Pestivirus* strains, according to the PNS genotyping method, allowed the identification of consensus motifs shared by all the *Pestivirus* species, genus specific base-pairings, and characteristic species and genotype specific PNS, respectively (Table 4). Ten PNS positioned in the V1 and V2 loci were characteristic for the genus. PNS consensus motifs shared by all species in the genus, as the bulge formed by two cytosine nucleotides in the V1 stem position eleven, are presented in Table 4 and schematically shown in Figure 1. Based on the divergence limit value of 9 bp for genotype determination [17], twenty-three genotypes within the BVDV-1 species have been identified, from 1a to 1.23. BVDV-2 accounted for six genotypes, from 2a to 2f. BDV was clustered into ten genotypes, from a to j. BVDV-3 and CSFV were less heterogeneous with four genotypes.

Secondary sequence construction, efficiently obtained by both available software, Genetyx and PNS [24], revealed a conserved palindromic structure in the genus species (Figure 1). Only in few cases the elaboration of secondary structure resulted slightly problematic. Some difficulties have been encountered due to atypical sequences, showing insertions in the variable loci, as the Chinese BVDV-3 ovine strains HN1559 [KU053497], HN1507 [KU563155], HN1568 [KU053499] and HN1564 [KU053498] (Shi *et al.*, unpublished) with U U, A A, G G or C C bulge insertion in the V1 locus. Similarly, BVDV-3 strains IndMDV18963/12 [KM201318] and IndBHA6604/12 [KM201307] showed deletions in V3 locus positions 5 and 6. The predicted secondary structures of the three variable loci were aligned for comparison of base pairings in the different positions (Table 5). Different base pairing combinations were identified for genotype characterization (BVDV-1 n 416; BVDV-2 n 66; BVDV-3 n 33, BDV n 99 and CSFV n 37), and considered for identification marker definition. Strains showing sequence identity at the level of the three variable loci or sharing non-relevant variations as G\*U or G-C (G:Y) were excluded (Table 6). The summary of strains clustering into genotypes of *Pestivirus* species is presented in Table 7. At the species level, the observed taxonomic status of the examined strains corresponded to the estimation obtained by phylogenetic trees constructed from the alignment with the representative strains from the identified genogroups (Figures 2-4). Different species genotypes, showing peculiar genetic



**Table 4.** Palindromic nucleotide substitutions (PNS) characteristic to the genus *Pestivirus* and the BVDV-1, BVDV-2, BVDV-3, BDV, *Pestivirus* I and CSFV species with related genotypes. The position of base pairings is defined by numbering from the bottom of the variable locus. BVDV-1 genotypes Locus Characteristic PNS markers. Determinative low variable positions (LVP) selected for sequence clustering into genotypes. PP: prevalent position; HVP: highly variable position. R = A or G; Y = C or U. Genotype name according Vilcek *et al.* [165] and subsequent authors in parenthesis.

Genus	Locus	Characteristic PNS markers
	V1	Absence in position 22—size of V1 21 bp (exception U); C C bulge in position 11; A-U in position 10 (exceptions A C, U U, G A and G G bulges); Y:G (U*G or C-G) in position 8 (exceptions U-A, G*U, C A, C C, A G and G G bulges, insertion A in correspondence of 8r); U-A in position 7 (exception G:Y, U*G, G A and A A bulges, insertion A in correspondence of 7r); A in position 6 (exception G); Y:G in position 5; U in position 5 right nucleotide (exceptions U-A, C, U C and U U bulges, insertion A in correspondence of 5r); G:Y in position 4 (exception G A and A C bulges)
	V2	GGGGY loop (V2 positions 10-12: V2/10 G:Y; V2/11 GG; V2/12 G) (exceptions V2/12: GG-GGGGGG-insertion G in V2 loop, absence-GGGY-deletion of G in V2 loop; V2/11: CG-GCGGY; V2/10: GG-GGGGG, AG-AGGGG, UU-UGGGU); Y:G in position 8 (exception A G and C A bulges, insertion A)
Species	Locus	Characteristic PNS markers
<b>BVDV-1</b>	V1	V1/21 Absence; U-A in position 15 (exception Y:G-U*G or C-G, A A, C A and U U bulges)
	V2	G:Y in position 5 (exceptions A-U, U-A)
	V3	G:Y in position 5 (exception Y:G, G A bulge) A in position 10 (exceptions G, U, A-U, U-A, G:Y, C-G or A C, A A, G A, U U, C C and G G bulges or absence)
<b>BVDV-2</b>	V1	A-U or A C bulge in position 20 (exceptions G:Y, C C or A A bulges, absence); A, G or U in position 21 (exception G G, absence); absence in V1/20 and 21 is limited to strain A3-118/US is not considered in software parameters due to confusion with BVDV1 definition
	V2	U-A or Y:G in position 6 (exception C A bulge)
	V3	A-U or A C bulge in position 7 (exception G:Y and AA bulge Chinese strains)
<b>BVDV-3 (HoBi group)</b>	V1	U-A in position 15 (exception CA BGD/ZS1 and BGD/ZS5)
	V3	G-C or G*U in position 3 (exception A-U or GA bulge BGDZS1); A-U in position 7 (exception G-C or A G, C U, U U and U C bulges); A in position 10 (exception UA, G)
<b>BDV</b>	V1	C-G or U*G in position 13; A-U in position 14 (exceptions U-A, G:Y and A A bulge); G:Y or A-U in position 15 (exceptions C U and A C bulges)
	V2	U-A, Y:G or A-U in position 5
	V3	U C and U U bulges or Y:G in position 7 (exceptions A-U, U-A, G:Y and C C, A C, C U and C A bulges)
<b>BDV-2 (Italian ovine isolates) tentative species</b>	V1	C-G or U*G in position 13; U-A or C A bulge in position 15
	V3	G:Y or G G bulge in position 8
<b><i>Pestivirus</i> I</b>	V1	C-G or U*G in position 13; C-G in position 14; G:Y or A-U in position 15 (exceptions C U and A C bulges)
	V2	G:Y in position 5
	V3	Y:G in position 7; U U or C U bulge in position 10

## Continued

CSFV	V1	U-A in position 13 (exception U*G or C-G; C A bulge)
	V3	U-A in position 2; C A or U-A in position 7; U or C in position 8 (exception A)
<b>BVDV-1 genotypes</b>	<b>Determinative LVP (V1/14; V2/7; V3/4)</b>	<b>Additional markers (PP, LVP, HVP)</b>
	<b>Genotype markers</b>	
<b>BVDV-1a</b>	YG, GY, AU	V1/12 GY/AU; V2/5 GC; V2/7 (exception C C bulge)
<b>BVDV-1b</b>	UA, RY, GC (b.1V2/7 AU; b.2V2/7 GY)	<b>b.1</b> V1/14 (exception G A bulge) V2/7 (exception A C bulge) V3/4 (exception AU) <b>b.2</b> V1/14 (exception A A ; C A and G A bulges)
<b>BVDV-1.3 (D)</b>	CG, AC, AU	(V1/14 exception C A and A G bulges)
<b>BVDV-1.4</b>	CG, AU, GC	V1/12 GY/AU V3/6 UA V3/4 (exception AU)
<b>BVDV-1.5 (I)</b>	CG, GY, GC	V3/2 AC
<b>BVDV-1.6 (N, T)</b>	CG, GC, GU	V1/16 AU/UU; V2/2 CG; V2/6 GY; V3/3 CG; V3/6 UA; V3/8 UC/AC/GC
<b>BVDV-1.7 (O)</b>	CG, GY, GC	V2/2 UA; V3/9 AU/AA/GC
<b>BVDV-1.8 (C)</b>	CG, GC, AU (Root A non A)	V1/12 AC (exception C C bulge) V2/7 (exception AU) V3/4 (exception AC)
<b>BVDV-1.9 (H)</b>	CG, GC, GC	V1/20 GA; V2/6 CU/UU
<b>BVDV-1.10 (Q)</b>	CG, GC, GC	V1/15 AA or CA (exception species marker); V2/2 UA; V2/5 GC; V3/9 UC/UU
<b>BVDV-1.11 (E)</b>	CG, GY, GC	V1/17 GA/GG/AG (exception A A bulge and GU); V2/6 AU/AC/UU; V3/6 UA; V3/8 AA/GA (exception A G bulge)
<b>BVDV-1.12 (F, R, S)</b>	<b>12.1</b>	<b>12.1</b>
	CG, GY, GC	V3/6 GC V3/8 AU/AC
<b>BVDV-1.13 (C, J)</b>	<b>12.2</b>	<b>12.2</b>
	CG, AU/GC, GC	V3/6 GA; V2/7 (exception GC)
<b>BVDV-1.14 (R)</b>	CG, GY, GC	(V1/14 exception C A bulge); V1/16 CR (C-G or C A bulge) V1/17 GA (exception G*U); V2/6 AU (exception A C bulge)
<b>BVDV-1.15 (M)</b>	YG, GY, AU (Root A non A)	V2/5 AU (exception species marker)
<b>BVDV-1.15 (M)</b>	<b>15.1</b>	<b>15.1</b>
	CG, GC/AC, GY/AU (Also Root A non A)	V1/19 GY/GG; V2/2 CG/UA; V2/4 CG/CA (exception G G bulge); V2/5 GC; V3/9 CC/UC/UU (exception U) V3/4 (exception UA)
<b>BVDV-1.15 (M)</b>	<b>15.2</b>	<b>15.2</b>
	CG, GC, GY/AU (Also Root A non A)	V1/19 GY/CU; V2/4 CG; V2/5 AU (exception species marker); V3/9 CC/UC

## Continued

<b>BVDV-1.16 (L)</b>	CG, GC, GC	V2/6 UA
<b>BVDV-1.17 (F)</b>	CG, GC, GC	V3/6 AA (exception G A bulge); V3/8 A A bulge
<b>BVDV-1.18 (P)</b>	CG, GC/AC, GC	V1/17 AU/CA V2/6 GC; V3/3 UA; V3/8 AA
<b>BVDV-1.19 (G)</b>	CG, GY, GC	V1/15 CG; V2/5 AU (exception species marker)
<b>BVDV-1.20 (L, X)</b>	<b>BVDV-1.20.1</b> YG, GY, AU (Root A non A)	<b>BVDV-1.20.1</b> V1/15 CG; V2/5 AU (exception species marker)
	<b>BVDV-1.20.2</b> YG, GY, GC	<b>BVDV-1.20.2</b> V1/15 CA; V2/5 AU (exception species marker)
<b>BVDV-1.21 (U)</b>	UA, GC, UA (origine B, unique)	<b>BVDV-1.21.1</b> V1/15 CG (exception species marker); V3/4 exception G A bulge; V3/5 CG (exception species marker); V3 loop position 10 CG (exception species marker) (exception C C bulge); V3 size longer (11bp - 21n) (exception 10bp - 20n); V3/2 AU
		<b>BVDV-1.21.2</b> V3/4 exception G A bulge; V3/5 CG (exception species marker); V3 loop position 10 CG (exception species marker) (exception C C bulge); V3 size longer (11 bp - 21n) (exception 10 bp - 20n) V3/2 GU
<b>BVDV-1.22 (K)</b>	<b>BVDV-1.22.1</b> CG, AC, GC	<b>BVDV-1.22.1</b> V2/3 UA; V2/5 GC
	<b>BVDV-1.22.2</b> CG, GC, GC	<b>BVDV-1.22.2</b> V2/3 UA; V2/5 UA (exception species marker)
<b>BVDV-1.23 (P)</b>	CG, AC, GC	V3/5 GA
<b>BVDV-2 genotypes</b>	<b>Locus</b>	<b>Characteristic PNS markers</b>
<b>BVDV-2a</b>	V1	C-G in position 16; U*G, C-G or U-A in position 18 (exceptions U C and C A bulges)
<b>BVDV-2b</b>	V1	G-C or G*U in position 12; U-A in position 16 (exception CA bulge); G A, G G or A C bulges or G-C in position 17; G G or G A bulges in position 18
	V3	higher V3 loop, U in position 10 (exceptions G*U, UA and A)
<b>BVDV-2c</b>	V1	U-A in position 8; G A bulge in position 17; G A bulge in position 18; C C bulge in position 20
	V3	G-C in position 7
<b>BVDV-2d</b>	V1	Shorter V1 loop-size of V1 19 bp; U-A in position 15; GA bulge in position 17; G A bulge in position 18
<b>BVDV-2e</b>	V1	G-C in position 12; C-G in position 14; C-G in position 16; G A bulge in position 17; G A bulge in position 18.
	V3	higher V3 loop, C in position 10
<b>BVDV-2f</b>	V1	A-U or A C bulge in position 9; A-U in position 12; U-A in position 16 (exception GA bulge); G A bulge in position 17; G A bulge in position 18
	V3	higher V3 loop, U or U-A in position 10
<b>BVDV-3 genotypes</b>	<b>Locus</b>	<b>Characteristic PNS markers</b>
<b>BVDV-3.1</b>	V1	A-U in position 1 (exception G:Y, U U or C U bulges); Y:G in position 8; U-A or C A bulge in position 15; A G, A A, U U or G A bulges, A-U or G:Y in position 18
	V2	Y:G in position 3
	V3	G:Y (exception G G bulge) in position 4; Y:G, A G or U-A in position 8

## Continued

<b>BVDV-3.2</b>	V1	G:Y in position 1; A-U in position 8; U-A in position 15; A G bulge in position 18
	V2	C A bulge in position 3
	V3	A C bulge in position 4; Y:G in position 8
<b>BVDV-3.3</b>	V1	A-U in position 1; Y:G in position 8; C A bulge in position 15; A A bulge in position 18
	V2	Y:G in position 3
	V3	U-A in position 4; G:Y in position 8
<b>BVDV-3.4</b>	V1	C C bulge in position 1; Y:G in position 8; U-A in position 15; C U bulge in position 18
	V2	Y:G in position 3
	V3	A-U in position 4; Y:G in position 8
<b>BDV genotypes</b>	<b>Locus</b>	<b>Characteristic PNS markers</b>
<b>BDV-a (1)</b>	V1	A-U or C U bulge in position 9; A A or A G bulges in position 18 (exception A-U and G G bulge)
	V2	A-U in position 1; A-U, Y:G or U-A in position 5
	V3	A A, G A or A C bulges in position 8 (exceptions G:Y, A)
<b>BDV-b (2)</b>	V1	A-U in position 9 (exception G:Y); U-A, Y:G in position 16
	V2	A-U in position 1; U-A in position 5
	V3	C U bulge in position 1; G:Y or U U bulge in position 2; A A bulge in position 8 (exception A-U)
<b>BDV-c (6, 8, SW)</b>	V1	G:Y in position 9; A-U, A G or A C bulges in position 16
	V2	A-U or A C bulge in position 1; U-A in position 5
	V3	A A bulge in position 8
<b>BDV-d (3)</b>	V1	AU in position 3; G:Y in position 9; A-U in position 12; A-U in position 14; U-A in position 16; C A, C U or U U bulges in position 19
	V2	G:Y in position 1; U-A in position 5
	V3	A G or A A bulges in position 8
<b>BDV-e (4)</b>	V1	G:Y in position 9; G:Y or G G bulge in position 18; G:Y or G G bulge in position 20
	V2	G:Y in position 1; Y:G in position 5 (shared with A only)
	V3	U-A or C A bulge in position 8
<b>BDV-f (4, 5, 7)</b>	V1	G:Y in position 9 (exception A-U); A-U in position 16 (exception G*U); Y:G, G:Y or G G bulge in position 18 (exception A G bulge)
	V2	G:Y in position 1 (exceptions A-U and C U or G G bulges); Y:G in position 5 (shared with A only)
	V3	U-A, Y:G, A A or C A bulges in position 8 (exception U C bulge)
<b>BDV-g (TU)</b>	V1	G:Y or A-U in position 9
	V2	A-U in position 1; A-U in position 5 (exception G:Y)
	V3	U-A in position 2 (g1 V3/2 CG—strains TU ITA sicily—Shared only with v60-bd-b); Y:G in position 7 (exception U-A—shared with a1 and e1); U or C in position 8
<b>BDV-h (3)</b>	V1	AU in position 3; G:Y in position 9; U-A in position 12; A-U in position 14; U-A in position 16; CC in position 19
	V2	G:Y or A-U in position 1; U-A in position 5
	V3	G:Y in position 4; A-U or A A bulge in position 8
<b>BDV-i</b>	V1	V1/8 CC/AG; G:Y in position 9 (exception GA bulge); Y:G in position 16; A-U or A G bulge in position 19

## Continued

BDV sub-genotypes	Locus	Characteristic PNS markers
	V2	A-U in position 1; U-A in position 5
	V3	G:Y in position 4; A A or C A bulges in position 8.
<b>BDV-j (3)</b>	V1	G:Y in position 3 (exception A-U); G:Y in position 9; G:Y in position 14 (exception A-U); U-A or Y:G in position 16; C C, U C bulges, U-A or Y:G in position 19
	V2	A-U in position 1; U-A in position 5
	V3	G:Y in position 4; Y:G, G:Y, U C or A A bulges in position 8
<b>BDV-a1 (1)</b>	V1	U right nucleotide in position 7; A-U in position 12; A-U or CU in position 15; AA or AG in position 18
<b>BDV-a2 (1)</b>	V1	G right nucleotide in position 7; G:Y or CC in position 12; G:Y in position 15; AG or GG in position 18
<b>BDV-a3 (1)</b>	V1	A right nucleotide in position 7; G:Y in position 12; AC in position 15; AG in position 18
<b>BDV-a4 (1)</b>	V1	G right nucleotide in position 7; G:Y in position 12; G:Y in position 15; A-U in position 18
<b>BDV-c1.1 (Sw)</b>	V1	A-U in position 16
	V2	A-U in position 1
	V3	G:Y in position 4
<b>BDV-c1.2 (8)</b>	V1	A-U in position 16
	V2	A-U in position 1
	V3	A-U in position 4
<b>BDV-c1.3 (8)</b>	V1	A C bulge in position 16; G:Y in position 21
	V2	A-U in position 1
	V3	G:Y in position 4
<b>BDV-c2 (6)</b>	V1	G:Y or U C and U U bulges in position 20; U or U U bulge in position 21
	V2	A C bulge in position 1
	V3	C C bulge in position 7 (exception U C bulge)
<b>BDV-d1 (3)</b>	V1	CU or UU in position 19
	V3	A-U in position 4; A G bulge in position 8
<b>BDV-d2 (3)</b>	V1	CA in position 19
	V3	G:Y in position 4; A A bulge in position 8
<b>BDV-e1 (4)</b>	V1	G:Y in position 16
	V3	U-A in position 8
<b>BDV-e2 (4)</b>	V1	A-U in position 16; G in position 21
	V3	CA in position 8
<b>BDV-f1 (4, 5)</b>	V1	G right nucleotide in position 7; CU in position 17; GG in position 18
	V2	G:Y or Y:G in position 2
	V3	UU or UC in position 7
<b>BDV-f2 (4)</b>	V1	U right nucleotide in position 7; Y:G in position 17; G:Y in position 18
	V2	UA in position 2
	V3	A-U or AC in position 7

**Continued**

<b>BDV-f3 (4)</b>	V1	G right nucleotide in position 7; CU or A-U in position 17; G:Y, GG or AG in position 18
	V2	UA in position 2
	V3	UU or UC in position 7
<b>BDV-f4 (4, 7)</b>	V1	G right nucleotide in position 7; CU or CC in position 17; Y:G in position 18
	V2	Y:G or C U bulge in position 2
	V3	U U or U C bulges in position 7
<b>BDV-g1 (TU)</b>	V1	G:Y in position 1
	V2	U-A, C-G or U*G in position 3
	V3	A-U or CU in position 6 (CU all strains from Sicily; shared only with B V60 and D2 AV only)
<b>BDV-g2 (TU)</b>	V1	A-U in position 1
	V2	A-U or AC in position 3
	V3	AC, UU or CC in position 6
<b>BDV-i1</b>	V1	C C bulge in position 8; A-U in position 19
	V3	C A bulge in position 8
<b>BDV-i2</b>	V1	A G bulge in position 8; A G bulge in position 19
	V3	A A bulge in position 8
<b>BDV-j1 (3)</b>	V1	A-U in position 3; A-U in position 14; CC in position 19
	V3	Y:G in position 8
<b>BDV-j2 (3)</b>	V1	G:Y in position 3; GC in position 14; UA or UG in position 19
	V3	G:Y, U C or A A bulges in position 8
<b>BDV-j3 (3)</b>	V1	G:Y in position 3; GC in position 14; UC in position 19
	V3	Y:G in position 8; V3/9 A
<b>CSFV genotypes</b>	<b>Locus</b>	<b>Characteristic PNS markers</b>
<b>CSFV-a</b>	V1	A C bulge in position 15 (exception G:Y and A-U)
	V2	U-A in position 5; G:Y in position 7
	V3	A-U in position 1 (exception A G bulge)
<b>CSFV-b</b>	V1	G:Y in position 15; A G bulge in position 19; U-A in position 20
	V2	A-U in position 5; A C bulge in position 7
	V3	A-U in position 1; U C bulge in position 6
<b>CSFV-c</b>	V1	A-U in position 15
	V2	U-A in position 5; G:Y in position 7
	V3	A G bulge in position 1
<b>CSFV-d</b>	V1	A C bulge in position 15
	V2	U-A in position 5; G:Y in position 7
	V3	U U bulge in position 1
<b>CSFV genotype variants</b>	<b>Locus</b>	<b>Characteristic PNS markers</b>
<b>CSFV-a1</b>	V1	AC in position 15
	V2	A-U in position 1; A-U in position 6

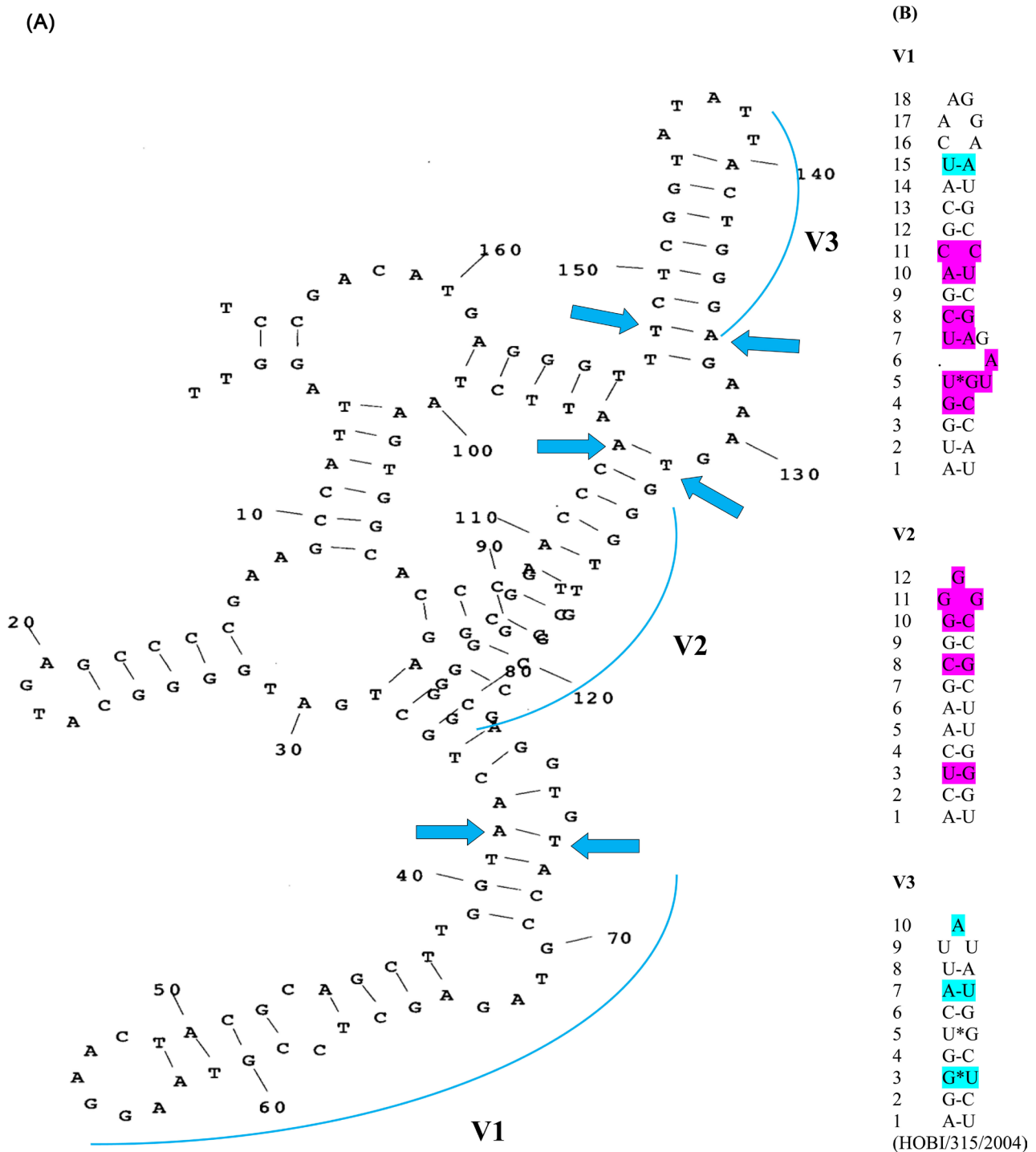
## Continued

	V3	C or U in position 8
CSFV-a2	V1	AC in position 15 (exception G-C)
	V2	A-U in position 1; G:Y in position 6
	V3	A-U in position 6; U or C in position 8
CSFV-a3	V1	AC in position 15
	V2	AC in position 1; G:Y in position 6
	V3	A in position 8
CSFV-a4	V1	G:Y in position 15
	V2	A-U in position 1; G:Y in position 6
	V3	AC or UC in position 6; A in position 8 (exception C)
CSFV-a5	V1	A-U in position 15
	V2	AC in position 1; A-U in position 6
	V3	U in position 8
CSFV-a6	V1	G:Y in position 15
	V2	A-U in position 1; A-U in position 6
	V3	C in position 8

characteristics, were restricted to specific geographic areas as in Turkey, China and other Asian countries (**Figure 5**).

The BVDV-1 species was heterogeneous. Some Asian strains showed exception at the level of BVDV-1 species markers. For example, Chinese strains of the genotype BVDV-1.10 showed divergence in V1/15 with A A or C A bulges, instead of species marker U-A pairing. In sub genotype 1.15.2, all strains showed species marker exception in V2/5, with A-U instead of G-C. Similarly, strain S153 [8], genotype 1.23, showed an atypical G A bulge at the level of species marker position in V3/5. The Asian clusters 1.8 and 1.15 shared a root characteristic of genotype BVDV-1a (V1/14 C-G, V2/7 G-C and V3/4 A-U). All the strains belonging to the group 1.8 showed an A C bulge in position 12 in V1 locus, a base pairing not present in any member in the genotype 1a. In this group have been included bovine strains from Australia, China and Japan as Bega (Mackintosh *et al.*, unpublished), Shitara/01/05 [28], Manasi [9], the Australian contaminant strain A1-114/AU [29] and the contaminant strain CRFK reported from Japan [30]. Among Chinese strains of the genotype 1.15, reported as genotype M [2] [7], root A was present in only the strain LZ05 of the sub genotype 15.1. In the sub genotype 15.2, characterized also by an exception at the level of species marker in V2/5 (A-U instead of G-C), root A was present in the majority of the strains. The genotype 1.21 showed a new and atypical U-A/G-C/U-A root associated to the genotype b (sub genotype 2). BVDV-1b, characterized by a variation of base pairing at the level of position 7 in V2 (b.1 V2/7 A-U; b.2 V2/7 G\*U or G-C), was the only other BVDV-1 genotype showing U-A pairing in LVP V1/14. The V1/14 U-A was shared exclusively with BVDV-1b and BVDV-1.21





**Figure 1.** Secondary structure of the entire 5'-UTR sequence of BVDV-3 strain Hobi/315/2004 [98] (a) predicted according to the algorithm of Zuker and Stiegler [22], using the Genetyx-Mac version 10.1 program package (Software Development Co., Ltd., Tokyo, Japan). The minimum free energy was calculated by the method of Freier *et al.* [23]. Minimum free energy:  $-88.51$  Kcal/mol. V1-V3 palindromic loci are identified in the 5'-UTR sequence. Variable loci starting base pairs (position 1) referred to PNS procedure are indicated by blue arrows. (b): Schematic presentation of V1-V3 palindromic loci in the 5'-UTR of the tentative species BVDV-3 strain Hobi/315/2004. Base pairings characteristic to the genus (PNS genus specific) are shown in violet. The characteristic base pairings of the species BVDV-3 (PNS species specific) are represented in blue. Distance of V3 from V2: 5 nucleotides. The position of base-pairings is defined by numbering from the bottom of the secondary structures. Watson-Crick base pairings are indicated by a dash (-); tolerated pairings in secondary structure are indicated by an asterisk (\*).



(Cont'd)

Variable locus	Position	V1	V2	V3	(*)
BVDV1	GC UA GC UG A UA CG GC GY CG	UA HV GA HV HV	UA GC AC	GC UA UC AA	
BVDV-1.16 (L)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	3
BVDV-1 TR72		AU CG	UA GC AC	GC UA UC AA	2
BVDV-1.17 (F)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	3
BVDV1 S		AU CG	UA GC AC	GC UA UC AA	3
BVDV1 J		AU CG	UA GC AC	GC UA UC AA	3
BVDV-1.18 (P)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	3/4.2
BVDV1 T106		AU CG	UA GC AC	GC UA UC AA	2
BVDV1 Camel isolate 5		AU CG	UA GC AC	GC UA UC AA	2
BVDV1 BJ0701		AU CG	UA GC AC	GC UA UC AA	3
BVDV-1.19 (G)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	3.50
BVDV1 A		AU CG	UA GC AC	GC UA UC AA	4
BVDV1 L		AU CG	UA GC AC	GC UA UC AA	3
BVDV-1.20 (L, X)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	5.75
BVDV-1.20.1(L, X)		AU CG	UA GC AC	GC UA UC AA	5.66
BVDV1 CH-01-08		AU CG	UA GC AC	GC UA UC AA	6
BVDV-1.20.2(L)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	6
BVDV1 PGI3a07		AU CG	UA GC AC	GC UA UC AA	5.37
BVDV-1.21 (U)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	3
BVDV-1.21.1(U)		AU CG	UA GC AC	GC UA UC AA	3
BVDV1 130/15-5364		AU CG	UA GC AC	GC UA UC AA	6
BVDV-1.21.2(U)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	5.71
BVDV1 m31182		AU CG	UA GC AC	GC UA UC AA	6
BVDV-1.22 (K)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	6
BVDV-1.22.1(K)		AU CG	UA GC AC	GC UA UC AA	3
BVDV1 CH-05-b1		AU CG	UA GC AC	GC UA UC AA	3
BVDV-1.22.2(K)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	6.75
BVDV1 SuwaCp		AU CG	UA GC AC	GC UA UC AA	7
BVDV-1.23 (P)	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	CG UA GC UG A UA CG GC GY CG	UA GC AC	GC UA UC AA	13
BVDV1 S153 (V2/8 CA; V2/10 GG)		AU CG	UA GC AC	GC UA UC AA	13

Variable locus	Position	V1	V2	V3	(**)
BVDV-2	GY UA GC UG A UA CG GC AU CG UA CG CG	UA HV GA HV HV	UA GC AC	GC UA UC AA	
BVDV-2A group					
BVDV-2a1.1	1 2 3 5r 6 7 8 9 12 13 14 15 16 17 18 19 20 21 22	GY UA GC UG A UA CG GC AU CG UA CG CG	UA GC AC	GC UA UC AA	0
BVDV-2 Lees		AU CG	UA GC AC	GC UA UC AA	0
BVDV-2 CPA		AU CG	UA GC AC	GC UA UC AA	1
BVDV-2 Parvo		AU CG	UA GC AC	GC UA UC AA	1
BVDV-2 MP		AU CG	UA GC AC	GC UA UC AA	1





(Cont'd)

Variable locus	V1										V2										V3																											
	1	2	3	5r	6	7	8	9	12	13	14	15	16	17	18	19	20	21	22	1	2	3	4	5	6	7	9	GC	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Position	GY	UA	GC	UG	A	UA	CG	GY	CG	UA	UA	UA	CA	AG	AG	-	-	-	-	1	2	3	4	5	6	7	9	GC	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Prevalent base pairs	AU	UA	GC	UG	A	UA	CG	GC	YG	AU	UA	CA	AG	AG	-	-	-	-	-	AU	CG	YG	CG	AU	AU	GC	GC	AU	GY	GY	GC	YG	CG	AU	UU	A	A	AU	GY	GY	GC	YG	CG	AU	UU	A	A	
<b>BVDV-3</b>																																																
<b>BVDV-3.1.1 variant 1</b>																																																
BVDV-3 Hob1/Brazil/200/2002	.																																															
BVDV-3 LV01/12	.																																															
BVDV-3 B2-42/A (v1/10 AC)	.																																															
BVDV-3 D32/00_Hobi (ins v1/9r.6)	.							GCG																																								
BVDV-3 F1-29/BR	.																																															
BVDV-3 A2/BR	.																																															
BVDV-3 E4/SA	.																																															
BVDV-3 B3/AU	.																																															
BVDV-3 G1-35/AU	.																																															
BVDV-3 B6/US	.																																															
BVDV-3 B4-3/CA	.																																															
BVDV-3 LPV-WR/BR11	.																																															
BVDV-3 Au/9SB007/09	.																																															
<b>BVDV-3.1.1 variant 2</b>																																																
BVDV-3 JS12/01																																																
<b>BVDV-3.1.1 variant 3</b>																																																
BVDV-3 Th/04_KhonKaen																																																
<b>BVDV-3.1.2</b>																																																
BVDV-3 HN1519	.																																															
BVDV-3 HN1524	.																																															
BVDV-3 HN1542	.																																															
BVDV-3 HN1537	.																																															
BVDV-3 HN1528	.																																															
BVDV-3 HN1513	.																																															
BVDV-3 HN1518	.																																															
BVDV-3 HN1559 (v1/1.2 UU)																																																
BVDV-3 HN1507 (v1/1.2 AA)																																																
BVDV-3 HN1568 (v1/1.2 GG)																																																
BVDV-3 HN1564 (v1/1.2 CC)																																																
<b>BVDV-3.1.3</b>																																																
BVDV3IndABI16023/12 (v2/10 UU)																																																
BVDV3IndABI16020/12 (v2/10 UU)																																																
<b>BVDV-3.1.4</b>																																																
BVDV-3 BGDZS1																																																
<b>BVDV-3.2</b>																																																
BVDV-3 IndMDV18963/12	GC																																															
BVDV-3 IndBHA6604/12	GC																																															
<b>BVDV-3.3</b>																																																
BVDV-3 BGDZS5																																																
<b>BVDV-3.4</b>																																																
BVDV-3 BGDZS3	CC																																															









**Table 6.** BVDV-1, BVDV-2, BVDV-3, BDV and CSFV species strains showing sequence identity at the level of the three variable loci.

Reference strain	Identical strains (non relevant variations)	Countries (number of sequences)	Host types (number of sequences)
<b>BVDV-1</b>			
06z71	06z127.	South Korea (2)	Cattle (2)
106/15	121/14-45.	Italy (2)	Cattle (2)
107/15	203/16-5461; 584/14-10; 584/14-11; 642/14; 810/13-15.	Italy (6)	Cattle (6)
124/11	331/15; 612/14; 640/14-681; 811/14.	Italy (5)	Cattle (5)
125 85	2703D 99; 2750A 99; 2586X 99; 4796 94; 80/1 ncp 82; H851 98.	Ireland (7)	Cattle (7)
130/15-5364	13015-4215 (V2/8 CG, V3/7 CG).	Italy (2)	Cattle (2)
133/02	12p (V2/3 UG); 3310/01; 4092/00; UEL9-BR/11.	Brazil (2); Spain (3)	Cattle (4); Human (1)
192/16	169/15; 245/14-30; 247/14-16; 285/16; 306/16; 332/15; 57/14-750; 583/14; 641/14-26; 641/14-3.	Italy (11)	Cattle (11)
1/A/00	2/A/00; 3/A/00.	Italy (3)	Cattle (3)
1/B/01	2/B/01; 3/B/01; 4/B/01; 5/B/01; 6/B/01; 7/B/01; 8/B/01; 1/C/01.	Italy (9)	Cattle (9)
1R	i36P; i6.89; ncp2.	Argentina (4)	Cattle (4)
2900/83	IT99-5279-1; K3-3/DK; LOG_ca_02.	Denmark (2); Germany (1); Italy (1)	Cattle (3); Contaminant (1)
2032/01	2708/01.	Spain (2)	Cattle (2)
23/16	24/16; IT99-4292-1.	Italy (3)	Cattle (3)
2343/01	1041/01 (V2/2 UG).	Spain (2)	Cattle (1); Sheep (1)
2B	17P; cb1; CH692; CHLP26; CHLP30.	Argentina (3); Chile (3)	Cattle (6)
3251/01	Lamspringe738.	Germany (1); Spain (1)	Cattle (2)
39/14	251/14; 88/14; 89/14.	Italy (4)	Cattle (4)
3/VR/95	4/VR/95 (V3/5 GU); 585/14 (V3/2 GC).	Italy (3)	Cattle (3)
4382/01	4629/01 (V2/7 GU).	Spain (2)	Cattle (2)
51/06	52/06.	Poland (2)	Cattle (2)
535/14	122/14-43; 694/14.	Italy (3)	Cattle (3)
5/VR/96	GH-Sarma; R3680/00.	Italy (1); Switzerland (2)	Cattle (3)
71-16	71-03.	France (2)	Cattle (2)
71982/2011/PA	71982/2011/2PA.	Italy (2)	Goat (2)

## (Cont'd)

7546	7548.		Sweden (2)	Sheep (2)
79/11	103/11.		Italy (2)	Cattle (2)
853/15	203/16-9326.		Italy (2)	Cattle (2)
86713	IT99-5982 (V1/18 CG, V2/7 GC); IT99-5986 (V1/18 CG, V2/7 GC); IT99-3290-6 (V1/19 GU).		Argentina (1); Italy (3)	Cattle (4)
Antila	Dorado.		USA (2)	Alpaca (2)
B1056	114 817.		UK (2)	Sheep (2)
BJ0701	BJ0703.		China (2)	Cattle (2)
BJ09_04	JS-05059.		China (2)	Cattle (2)
BJ1302	BJ1303.		China (2)	Cattle (2)
CD89	PT16-03.		Belgium (1); Portugal (1)	Cattle (2)
CH615	GH113.		Chile (2)	Cattle (2)
CHL1086	C2-146/US (V3/2 GC); CHL1025; CHL1061; CHL1071; CHL1076; CHL1092; CHL1109; CHL917; CHL918 (V3/2 GC); CHL919 (V3/2 GC); CHL920.		Chile (11); USA (1)	Cattle (11); Contaminant (1)
CHL379	CHL184.		Chile (2)	Cattle (2)
CHL921	CHL1068.		Chile (2)	Cattle (2)
CHLP22	CHL1078; CHL1087; CHL927; CHL928; CHL939; CHL971; CHL972; CHL992; CHLP23; CHLP24; CHLP31; CHLP32; CHLP33.		Chile (14)	Cattle (14)
CH-R2064/93	Mother 2 R2064/93.		Switzerland (2)	Cattle (2)
CP1874	Bov/Ita/856.14-578; IT99-4250; IT99-5212-5; IT99-5212-2; IT99-5212-1; PT45-04.		Belgium (1); Italy (5); Portugal (1)	Cattle (7)
CP1945	51/15; 385/15.		Belgium (1); Italy (2)	Cattle (3)
Culi4	YVD947.		Belgium (2)	Cattle (2)
Culi6	10A/LC/97; 4325/01; IT99-5555-3; IT99-5555-4; PT14-03; ZVD278.		Belgium (2); Italy (3); Portugal (1); Spain (1)	Cattle (7)
D1120/1	D1432/P.		UK (2)	Sheep (2)
Deer	S-ALT5/K.		South Africa (1); UK (1)	Cattle (1); Deer (1)
E4-168/SA	PT32-03.		Portugal (1); South America (1)	Cattle (1); Contaminant (1)
Europa	BRU*0615; IT99-5707.		Belgium (1); Italy (1); Japan (1)	Cattle (1); Contaminant (1); Human (1)
F	58/14; H.		Austria (2); Italy (1)	Cattle (3)
F1-4/BR	CH565; Tunisia 294.		Brazil (1); Chile (1); Tunisia (1)	Cattle (2); Contaminant (1)

(Cont'd)

Reference strain	Identical strains (non relevant variations)	Countries (number of sequences)	Host types (number of sequences)
<b>BVDV-1</b>			
FLK	2110C; 246/14-22; 368/02; HeLa; Ind 446; IndS1168; IndS1171; IndS1181; IndS1455 (V1/9 GU); MOLT-4; MRH1375; NGR2; NGR3; NGR11; NGR12; PT17-03; Q713; WiDr.	Belgium (1); Canada (1); India (5); Italy (1); Japan (3); Mexico (4); Portugal (1); Spain (1); UK (1); USA (1)	Cattle (11); Contaminant (8)
G	221/10; B306/06.	Austria (2); Italy (1)	Cattle (3)
GXBH-EB34	JS-03148; JS-03198.	China (3)	Buffalo (1); Cattle (2)
HH	H503.	Japan (2)	Contaminant (2)
HZ05	TJ0801.	China (2)	Cattle (2)
i13	424/13 (V1/19 GC); 66.1; 66.6; 749/13 (V21/19 GC); i53.	Argentina (4); Italy (2)	Cattle (6)
i89	i393.	Argentina (2)	Cattle (2)
IS25CP01	IS2601ncp.	Japan (2)	Cattle (2)
isolate 6 (Camel)	isolate 7 (Camel).	China (2)	Bactrian camel (2)
IT99-4277	IT99-5964-2; IT99-6507; IT99-6122; IT99-4274.	Italy (5)	Cattle (5)
IT99-4859	IT99-4302; IT99-4680; IT99-4190.	Italy (4)	Cattle (4)
IT99-7101	IT99-7162 (V3/4 GU); IT99-6869 (V3/4 GU); IT99-7164; IT99-7221 (V3/4 GU).	Italy (5)	Cattle (5)
J	R.	Austria (2)	Cattle (2)
JE	0192; No. 12; A014; C009T; FU411; HC725; HE726; HE728; IQ19A; KQ25A; KQ25B; LQ28A (V1/9 GU). KS86-1cp.	Japan (14)	Cattle (2); Contaminant (12)
JS-3094	GXBH-EB20; GXBS-LB3; GXBH-EB33; GXHZ-JB24; GXLZ-BB5; GXLZ-BB6; GXYL-KB22; GXYL-KB53; HB-0244; HB-0273; HB-050030; HB-050057; HB-090913; JS-01159; JS-04198; LN317-6; NMG311-3; NMG312-26; NMG312-32; NMG313-28; NMG313-35; NMG313-55; NMG314-22; NMG314-51; NMG314-65; NMG315-1; QHQL-216; QHQL-225; QHQL-268.	China (31)	Buffalo (8); Cattle (20); Yak (3)
K7-5/NI	PT1-01; UGE_ca_62.	Denmark (1); Portugal (1); nd (1)	Cattle (2); Contaminant (1)
KA91	E5-158/US; H3-193/US; K2-1/CO.	Colombia (1); Japan (1); USA (2)	Cattle (1); Contaminant (3)
KM	Kyj.	Slovakia (2)	Cattle (2)
Lamspringe 735	I0Jf-SKR; C1-142/US; B1I023; B1I201; cell-con-1; OK1(CA)NCP/03.	China (3); Germany (1); Japan (1); South Korea (1); USA (1)	Cattle (6); Contaminant (1)
LN-1	W4-1.	China (2)	Cattle (2)

## (Cont'd)

LN309-5	GXBS-LB8; GX CZ-FB7; GX CZ-FB25; GXHZ-JB11; GX LZ-BB4; HB-090166; JS-0197; JS-04138; JS-02007; JS-04119; JS-05002; JS-99054; JS-X02126; NMG311-20; NMG315-5.	China (16)	Buffalo (5); Cattle (11)
LN311-17	LN311-25.	China (2)	Cattle (2)
M065B/93	M079B/91.	South Africa (2)	Cattle (2)
Manasi	Letuyi; Shihezi 148.	China (3)	Cattle (3)
Massimo 1	1891/99; 228/02; Culi 1; Massimo 2.	Belgium (1); Germany (1); Italy (1); Spain (1); Switzerland (1)	Cattle (3); Contaminant (2)
MDBK	U937.	Japan (2)	Contaminant (2)
M17IN/95	B463/06; CH-04-01b; CH-04-B2; UMM/126/07.	Austria (1); Chile (2); Italy (1); South Africa (1)	Cattle (5)
NADL	Bov/Ita/124.15-2; Bov/Ita/124.15-7; Bov/Ita/124.15-12; Bov/Ita/124.15-28; Bov/Ita/124.15-30; Bov/Ita/124.15-37; Bov/Ita/124.15-48; CH888; CHL1091.	Chile (2); Italy (7); USA (1)	Cattle (10)
Oregon	25H; 318; 65.2; R193572.	Argentina (3); Brazil (1); USA (1)	Cattle (4); Contaminant (1)
P	4050/00 (V1/12 GU).	Austria (1); Spain (1)	Cattle (2)
PT11-03	PT12-03.	Portugal (2)	Cattle (2)
S	MV98CB95; W (V1/18 GC).	Austria (2); South Africa (1)	Cattle (3)
S21	76865.	Argentina (1); China (1);	Cattle (2)
SD0803	11N36; BJ1022; BJ1123 (V1/5 CG); FJ1003; S53; ZJ1003.	China (7)	Cattle (4); Pig (3)
Singer	BVR1199; Ln 68.	Belgium (1); Ireland (1); USA (1)	Cattle (3)
SuwaCp	SuwaNcp.	Switzerland (2)	Cattle (2)
TFB	TFB2.	Argentina (2)	Cattle (1); Contaminant (1)
Vero	CV-1; MDCK.	Japan (3)	Contaminant (3)
XA08	HZ0601; NX0801; NX0802 (V1/9 G*U); NX0803; S12; S13; S22; S81 (V2/7 GU); S101; ZD05.	China (11)	Cattle (11)
XZ-141	NMG311-2.	China (2)	Cattle (1); Yak (1)

(Cont'd)

Reference strain	Identical strains (non relevant variations)	Countries (number of sequences)	Host types (number of sequences)
<b>BVDV-1</b>			
XZ-24	HB-050062; HB-080141; HB-080146; HB-090268; LN309-9; NMG313-1; NMG314-60; QHHY-22; QHTJ-303887; XZ-103; XZ-109; XZ-133; XZ-176; XZ-176; XZ-25; XZ-48; XZ-69; XZ-71; XZ-84; XZ-86; XZ-92.	China (21)	Cattle (7); Yak (14)
XZ-70	24/15 (V3/4 GU); 42M (V3/4 GU); Buffalo 2 (V3/4 GU); HB-060085 (V3/4 GU); HB-0134; HB-0258; HB-0288; HB-0899; Influenza2; IT99-5721 (V3/4 GU); LN311-15; LN311-27; LN313-15; LN314-19; LN311-34; M346T96; QHHY-21; QHQL-126; QHQL-219; QHQL-271; QHQL-292; QHQL-313; QHQL-321; QHQL-336; QHQL-337; QHTJ-L332; QHMY-N5; QHTJ-291; QHQL-295; QHQL-297; QHQL-299; QHMY-N6; QHQL-328; QHQL-340; QHQL-385; QHTJ-N303868; T(V3/4 GU); U(V3/4 GU); XZ-117.	Argentina (2); Austria (2); China (33); Italy (1); South Africa (1); Switzerland (1); UK (1)	Buffalo (1); Cattle (16); Contaminant (1); Yak (23)
ZVR711	1248/01.	Belgium (1); Spain (1)	Cattle (2)
<b>BVDV-2</b>			
34b	nep7.	Argentina (2)	Cattle (2)
63	098; 119.	Tunisia (3)	Sheep (3)
97/730	BM01 isolate 11.	NewZealand (1); Tunisia (1)	Cattle (1); Sheep (1)
Bov/Ita/232.03	Bov/Ita/187.15-38; CpIta124.15-20; CpIta124.15-23; CpIta190.15-36; CpIta191.15-21; OvIta188.15-18; OvIta189.15-69.	Italy (8)	Cattle (2); Goat (4); Sheep (2)
BSE1239	UVR420.	Belgium (2)	Cattle (2)
BSE341	E5-160/US.	Belgium (1); USA (1)	Cattle (1); Contaminant (1)
CPA	BSE92I; CPAE; EBTr.	Belgium (1); Japan (3)	Cattle (1); Contaminant (3)
Giessen1	AF112; B45-5; B50-5; B5-4; BA/35/02 (insertion C in V2 loop); BS-95-II; LO/04/04; LO/08/04; LO/13/04 (all LO strains with insertion C in V2 loop).	Germany (5); Italy (5)	Cattle (10)
i628	i4083; i61380.	Argentina (3)	Cattle (3)
Kosice	17583-97; 23025; 37Gr; CH515; CH649; CH693; CH809.	Austria (1); Chile (4); Slovakia (1); USA (2)	Cattle (8)



**(Cont'd)**

Munich 1	104/98; 4-5174; B52-2; Munich 2.	France (1); Germany (4)	Cattle (5)
NY93	15-103; Q126.	Canada (1); France (1); USA (1)	Cattle (3)
Lees	167 237; 168 149; 173 157; 175 375 (all with V1/9 GC).	UK (5)	Sheep (5)
Ov/Ita/182.15-33	Ov/Ita/189.15-97; Ov/Ita/191.15-16; Ov/Ita/192.15.	Italy (4)	Sheep (4)
OY89	SW90; SY-89; TC Shinozaki.	Japan (4)	Cattle (4)
Parvo	HE727; MMR-T; Rubella.	Japan (4)	Contaminant (4)
PT2-01	PT7-02; Ptn3.	Portugal (3)	Cattle (3)
S143	SD-130L.	China (2)	Cattle (1); Contaminant (1)
S172	S51.	China (2)	Contaminant (2)
SD-06	XJ-04.	China (2)	Cattle (2)
VS-260	VM96.	Brazil (2)	Cattle (2)
WG4622	IT-1732.	Italy (1); Netherland (1)	Contaminant (2)
<b>BVDV-3</b>			
Hobi/Brazil/200/2002	G2/BR (V2/3 U*G); H3-3/US; Hobi/Brazil/315/2004; Italy-83/10-cp; Italy-83/10-ncp; LV02/12; LV04/12; PB22487.	Brazil (6); Italy (2); USA (1)	Cattle (7); Contaminant (2)
IndABI16023/12	IndABI15987/12; IndABI15385/12 .	India (3)	Cattle (1); Zebu (2)
IndMDV18963/12	IndBHA6610/12; IndBHA6603/12; IndBHA6332/12; IndBHA6229/12; IndBHA6060/12; IndBHA5500/12; IndBHA5426/12; IndBHA5309/12; IndBHA5296/12; IndNAR0116/12; IndNAR0115/12.	India (12)	Zebu (12)
LV01/12	Au/A55110-1162/09; B1/AU; B5-3/MX; H1-50/AU; Italy-1/10-1; Italy-280/11-A; IZSPLV_To; LV03/12; SV478/07.	Australia (3); Brazil (3); Italy (3); Mexico (1)	Cattle (5); Contaminant (5)

(Cont'd)	Reference strain	Identical strains (non relevant variations)	Countries (number of sequences)	Host types (number of sequences)
<b>BDV</b>				
170-337	V2377/12, V2536/2.		UK (3)	Sheep (3)
2112/99	79248/01 (V2/7 GU), 80582/01, 87877/01.		Spain (4)	Sheep (4)
33S	Lot21, SN3G.		Tunisia (3)	Contaminant (3)
37A	35, 35T, RM, SN1T, SN2T.		Tunisia (6)	Contaminant (6)
8320-31NZ	90/8320/31, 91/5809.		New Zealand (1); UK (2)	Sheep (3)
90-F-6227	90-F-6338, 90-F-6339 (V2/2 CG, V2/7 GU), MA783513.		France (3); Italy (1)	Sheep (4)
90-F-6335	06-F-0299/369, 06-F-0299/420, 06-F-0299/477, 91-F-7014, 94-F-7446/1, 94-F-7446/2.		France (7)	Sheep (7)
93-F-7289	96-F-7624.		France (2)	Sheep (2)
A841/1	A1263/2, Q1488/1, Q1488/6.		UK (4)	Sheep (4)
AH12-01	AH12-02.		China (2)	Goat (2)
ARAN-1	AND-1, AND-2, AND-3, ARAN-2, ARAN-3, ARAN-4, ARAN-5, ARAN-6, ARAN-7, ARAN-8, ARAN-9, ARAN-10, ARAN-11, ARAN-12, ARAN-13, ARAN-14; RIBAGORCA-2; RIBAGORCA-3; RIBAGORCA-5.		Andorra (3); Spain (17)	Pyrenean chamois (20)
AV	85-F-488; BD/91.		France (2); Italy (1)	Sheep (3)
BT2305	Chemnitz (V1/18 UG), Stolpe (V1/18 UG, V2/6 GU).		Germany (3)	Sheep (3)
C121	C290 (V1/19 CG).		Spain (2)	Sheep (2)
CADI-1	CADI-2, CADI-3, CADI-4, CADI-5, CADI-6, CADI-7, CADI-8, CADI-9, CADI-10, CADI-11, CADI-12, CADI-13.		Spain (13)	Pyrenean chamois (13)
Chamois1	Chamois-Spain02; PALLARS-2; PALLARS-4; PALLARS-5; PALLARS-6; PALLARS-7 (all strains PALLARS V2/2 CG; V2/7 GU).		Andorra (1); Spain (6)	Pyrenean chamois (7)
Coos Bay 5 c	Coos Bay 5 nc.		USA (2)	Sheep (2)
ESP-97	ESP97-2, ESP97-3, ESP97-4, ESP97-5.		Spain (5)	Sheep (5)
FNK2012-1	FNK2012-2, FNK2012-3, FNK2012-4.		Japan (4)	Pig (4)

## (Cont'd)

FRESER-2	FRESER-3.	Spain (2)	Pyrenean chamois (2)
G1305	137/4.	UK (2)	Sheep (2)
Gifhorn	Gifhorn-sh.	Germany (2)	Pig (1); Sheep (1)
Italy-58987	Chamois-VdA-3.	Italy (2)	Alpine Chamois (2)
J1004	LA1108.	Germany (2)	Sheep (2)
LA/5909/09	LA/8567/14, MA/14123/12.	Italy (3)	Sheep (3)
M3	BDV-pig-SP-2007 (V1/1 GC; V/27 GC); VFMIII.	Spain (3)	Pig (1); Sheep (2)
Moreduunncp	T1802/1.	UK (2)	Sheep (2)
Orlu-S24	CERDANYA-1, CERDANYA-2, CERDANYA-3, CERDANYA-4, isard4606, Orлу-Etagne, Orлу-ORL 2004 02 C, Orлу-R36, Orлу-S36.	France (6); Spain (4)	Pyrenean chamois (10)
Rentier Rudolph	Wisent Casimir.	Germany (2)	Reindeer (1); Wisent (1)
Rocco	BU1-C3, BU1-C4, BU-1CRA22.	Spain (4)	Sheep (4)
T1789/1	V3196/1.	UK (2)	Sheep (2)

(Cont'd)	Reference strain	Identical strains (non relevant variations)	Countries (number of sequences)	Host types (number of sequences)
CSFV				
17-93	Wingene.		Belgium (1); Poland (1)	Pig (2)
322/MIB/2013	179/MIB/2014; 23/MIB/2014 (V1/1 GU); 99/MIB/2014.		India (4)	Pig (4)
39	Bangalore Ind-163/07; BV-P; CSFV-GZ-2009; CSFV-PK15C-NG79-11; CSFV/IVRI/VB-131; CSFV/1.1/dp/CSF0382/XXXX/Koslov (V2/7 G*U); CSF/MZ/KOL/73; CSF/MZ/SAI/76; HCVCAD22/14; Ind-173/08; Ind-174/08; Ind-175/08; Ind-176/08; Ind-239/08; Ind-243/08; Ind-272/08; NFP/AS-1; NFP/ML-2; NFP/ML-4; LK-VNIVViM (V1 GC); Rovac; SWH.		China (3); Czech Republic (1); Hungary (1); India (16); Russia (1); USA (1)	Contaminant (2); Pig (19); Vaccine (2)
Alfort	CSFwbFR5720040964-03; CSFwbFR6720030647-19; CSFwbFR6720050018-06; CSFwbFR6720050125-05; CSFwbFR6720050238-02; CSFwbFR6720060199-01; CSFwbFR6720070192-01; CSFV/2.3/wb/XXX0609/2004/Uelzen.		France (1); Germany (8)	Pig (8); Wild boar (1)
Alfort 187	39, Alfort A19, C strain (V1/1 GC), CAP (V1/1 GC), cF114, CSFV212L-13; Eystrup, GPE (-), KC (V1/1 GC), Hokkaido/66 (V1/1 GC), LOM, Miyazaki/81, Nakamura/66, Shimen, Vac A (V1/1 GC), Yamanashi/69.		China (4); France (2); Germany (1); India (1); Japan (6); Russia (1); Switzerland (1); USA (1)	Pig (14); Vaccine (3)
CSFV/2.3/SRB/126 4/ 2005	CSFV/2.3/dp/CSF0821/2002/HR/Novska; CSFV/2.3/dp/CSF857/2006/Borken.		Croatia (1); Germany (1); Serbia (1)	Pig (3)
CSFV/2.3/wb/CSF 1046/2009/Hennef	CSFV/2.3/wb/XXX0608/2005/Euskirchen.		Germany (2)	Wild boar (2)
Ibaraki/81-115	Ibaraki/81-20, Ibaraki/81-38, Ibaraki/81-40.		Japan (4)	Pig (4)
Okinawa/86	Okinawa-86-2.		Japan (2)	Pig (2)
Osaka/51	Osaka/71, Shizuoka/73.		Japan (3)	Pig (3)
Pader	HNLV-2011(V2/6 GU); HNSD-2012 (V2/6 GU); GD192011; Switzerland 2/93' (V1/1 GC), Switzerland 3/93/1', Switzerland 3/93/2', Venhorst, VRI4762 (V2/6 GU).		China (3); Germany (1); Malaysia (1); Netherlands (1); Switzerland (3);	Pig (9)
Switzerland 1/93	CSFV/2.3/SRB/6168/2006 (V1/1 GC); Switzerland 4/93'.		Serbia (1); Switzerland (2)	Pig (3)

**Table 7.** Clustering into genotypes of *Pestivirus* BVDV-1, BVDV-2, BVDV-3, BDV, *Pestivirus* I and CSFV species strains evaluated according to the Palindromic nucleotide substitution (PNS) method at the 5' untranslated region of RNA.

Species	Genotypes	Strains	Distance within species
BVDV-1			
BVDV-1a		<p><b>NADL, Oregon, SD-1; Singer, Weybridge;</b> 0192; 114 817; No. 12; 125 85; 15-3; 17-112; 1 77; 1R; 1R93; 20-V661-2; 2191; 2204/82; 2430 95; 252 84; 0.83</p> <p>2586X99; 25H; 26-1; 2703D99; 2750A 99; 28/1; 2L91; 3114 93; 318; 3187V; 371 89; 38376; 477194; 4796 94; 4898 94; 4H; 65.2; 66.3; 66.5; 68.883;</p> <p>80/1 cp 82; 80/1 ncp 82; 8087 99; 819 85; 985 84; A014; A3-116/US; A553; akT1; B1056; B2-175/AU; B551 98; BJI1308; Bov/Ita/124.15-2;</p> <p>Bov/Ita/124.15-7; Bov/Ita/124.15-12; Bov/Ita/124.15-28; Bov/Ita/124.15-30; Bov/Ita/124.15-37; Bov/Ita/124.15-48; BR275; BVRI199; C009T;</p> <p>CH888; CHL1068; CHL1091; CHL916; CHL921; CHLP28; DI120/1; D1-155/US; D1432/P; D771/1; D861; E3-177/NZ; E4-168/SA; FU411;</p> <p>H1-181/AU; H503; H686 98; H85198; HC725; HE726; HE728; HH; HN01; i36P; i6.89; i720; isolate 1; isolate 2; IQ19A; JE; JI-2/ZA; K869 98;</p> <p>K586-1cp; KQ25A; KQ25B; L1000 98; L1305; L322 98; Ln 68; LQ28A; M169B/93; M388A/90; MMR-K; ncp2; Nose; Pe515; PT32-03; PT33-03;</p> <p>PT35-03; PT38-03; PT41-03; Q1161/1; Q1161/2; R193572; SE1015; SH1060; S-V063W/95; TK-87-2; Trangie Y546; TYCP/91; VM.</p>	
BVDV-1b		<p><b>BVDV-1b1: Draper, Sanders;</b> 106/15; 107/01; 107/15; 10A/LC/97; 1103/88; 121/14-45; 12p; 122/14-43; 124/11; 133/02; 1946/01; 1/A/00; 2032/01; 203/16-5461; 21110C; 2218/01; 246/14-22; 2708/01; 2/A/00; 3291-97-A; 3310/01; 3311/15; 3417/00; 3425/01; 368/02; 379/15; 385/15; 3/A/00; 4050/00;</p> <p>4092/00; 4171/00; 4325/01; 51/15; 535/14; 551/02; 561/01; 584/14-10; 584/14-11; 605/12-54; 612/14; 640/14-681; 642/14; 694/14; 720/02; 7535;</p> <p>76865; 810/13-15; 811/14; 813/14; 832/01; 9189; A4-123/US; Antila; CH565; CHL184; CHL379; BJ09_04; BO2340/01; Buffalo 113/03; CD89;</p> <p>CP1872; CP1887; CP1945; Cullif6; CV-1; D; Dorado; F1-4/BR; FLK; FS720; HeLa; Ind446; IndS1166; IndS1170; IndS1171;</p> <p>IndS1181; IndS1222; IndS1455; IT99-4292-2; IT99-5555-3; IT99-5555-4; IT99-7161; JS-05059; K4-4/DO; MDBK; MDCK; MOLT-4; MRU1375;</p> <p>Mumps; NGR2; NGR3; NGR11; NGR12; P; PT11-03; PT12-03; PT14-03; PT16-03; PT17-03; PT21-03; PT30-03; PT31-03; PT37-03; PT40-03;</p> <p>PT44-04; Q713; S21; SON_nb2_02; TAR_nb_03; TGAC; TGAN; Tunisia 294; U937; UEL9-BR/11; Vero; Wi-38; WiDr; YVD947; ZVD278.</p>	0.63
BVDV-1b2		<p><b>NY-1; Osloss;</b> 10846/91; 1190V97; 1248/01; 169/15; 192/16; 2318/01; 24/15; 245/14-30; 247/14-16; 278/15; 285/16; 306/16; 3251/01;</p> <p>332/15; 3336/00; 3340/01; 3478/00; 3499/00; 3P; 4283/00; 42M; 424/13; 4382/01; 4629/01; 5284/00; 5551/84; 57/14-750; 583/14; 641/14-26; 641/14-3;</p> <p>66.1; 66.6; 7546; 749/13; 851/15; 86713; A5-128/US; Buffalo 2; B5-132/MX; Bov/Ita/124.15-43; Bov/Ita/856.14-578; CH113; CH226; CH615;</p> <p>CHL1025; CHL1061; CHL1071; CHL1076; CHL1086; CHL1092; CHL1109; CHL914; CHL917; CHL918; CHL919; CHL920; CP1874; CP1940;</p> <p>C2-146/US; E1-200/CA; E5-158/US; H2-190/MX; H3-193/US; HB-0134; HB-0258; HB-0288; HB-060085; HB-0899; HB-090219; i13; i297; i393;</p> <p>i467; i53; i63; i66.2; i736; i867; i89; Influenza2; isolate 12; IT99-4250; IT99-4274; IT99-4277; IT99-5163; IT99-5212-1; IT99-5212-2; IT99-5212-5;</p> <p>IT99-5274; IT99-5721; IT99-5964-2; IT99-5982; IT99-5986; IT99-6122; IT99-6506-32; IT99-6507; IT99-7400; K1-4/CA; K2-1/CO; K5-1/FR;</p> <p>K6-4/MX; KA91; Lamspringe738; LN311-15; LN311-17; LN311-25; LN311-27; LN311-34; LN313-15; LN314-19; M346T96; Marloie; PT19-03;</p> <p>PT23-03; PT25-03; PT45-04; PT8-03; PT9-03; QHHY-21; QHMY-N5; QHMY-N6; QHQL-126; QHQL-219; QHQL-271; QHQL-292; QHQL-295;</p> <p>QHQL-297; QHQL-299; QHQL-311; QHQL-313; QHQL-321; QHQL-328; QHQL-336; QHQL-337; QHQL-340; QHQL-345; QHQL-385;</p> <p>QHQL-405; QHTJ-17; QHTJ-291; QHTJ-L332; QHTJ-N303868; QHTJ-N304810; U; T; TFB; TFB2; XZ-117; XZ-70; YVR2394; ZVR711.</p>	

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<b>BVDV-1.3 (D)</b>	<b>Europa</b> ; 10J-SKR; 11; 128/88; 132/13; 16-111; 16484/93; 1/B/01; 1/C/01; 203/16-9326; 2900/83; 2/B/01; 3479-97-1; 3596/86; 3/B/01; 3/VR/95; 4/B/01; 4/VR/95; 58/14; 585/14; 5/B/01; 6/B/01; 71982/2011/PA; 71982/2011/2PA; 7/B/01; 8/B/01; 853/15; 9466/91; BRU*0615; B1-1/AU; B4-139/CA; BJ1023; BJ1120; BJ1201; C1-142/US; cell-con-1; F; H; IR-Isfahan-10; IT99-4190; IT99-4302; IT99-4680; IT99-4859; IT99-5707; IT99-5279-1; K3-3/DK; K7-5/NI; Lamspringe 735; LOG_ca_02; OK1(CA)NCP03; PT1-01; PT10-03; PT18-03; PT810; Reindeer2; SE5572; SE5726; Serow; SH9/11; STE_nb_02; SVL_ca_02; TRS_ca_02; UGE_ca_62; VIB_fe_93; Vkl.	0.57
<b>BVDV-1.4</b>	<b>438/02</b> ; CP1885; Egy/Ismailia/2014; Massimo 4; PT42-03.	0
<b>BVDV-1.5 (I)</b>	<b>23-15</b> ; 1041/01; 23-13; 2343/01; PT43-04.	0.75
<b>BVDV-1.6 (N)</b>	<b>so CP/75</b> ; 06z127; 06z71; KB01; S133; Shitara/02/06; SI/207/12; TY05.	0.86
<b>BVDV-1.7 (O)</b>	<b>IS25CP/01</b> ; AQGN96BI5; BJ09_24; IS26/01ncp; isolate 9; JS10116; S121; S43.	1.28
<b>BVDV-1.8 (C)</b>	<b>CRFK</b> ; A1-114/AU; AQMZ02AI21/2; Bega; Bega-like; CE1a10 (169/CA/10); isolate 3; isolate 4; Letuyi; Manasi; S183; Shihezi 148; Shitara/01/05.	1.90
<b>BVDV-1.9 (H)</b>	<b>KM</b> ; G; 221/10; 23/16; 24/16; 5/Vr/96; 812/14; B206/05; B306/06; B335/06; B463/06; Bov/Ita/124.15-14; bt437gamse; CH-04-01b; CH-04-B2; CH-R2064/93; CH-Sarma; IT99-4292-1; Kyj; M1096-16IN/95; M1096-5IN/95; MI7IN/95; Mother 2 R2064/93; R3680/00; UM/126/07.	1.46
<b>BVDV-1.10 (Q)</b>	<b>SD0803</b> ; 11N36; BJ1022; BJ1123; BJ1301; Camel6; FJI1003; isolate 6; isolate 7; JL1001; S53; S83; ZJ1003; ZJ1005.	1.86
<b>BVDV-1.11 (E)</b>	<b>10-84</b> ; 1372/01; 14-102; 1891/99; 196/14; 228/02; 251/14; 2555/01; 26-V639; 3186V6; 39/14; 4163/00; 88/14; 89/14; 8-Fr; 9-77; B248/06; Carlito; CH-Maria; Culi 1; E2-165/EU; IT99-3755; IT99-4258; IT99-5274-5; IT99-5279-2; IT99-5562; IT99-6869; IT99-7101; IT99-7162; IT99-7164; IT99-7221; L256; Massimo 1; Massimo 2; Ov/Ita/948.15-43; PT15-03; PT20-03; PT22-03; S182.	1.58



(Cont'd)	Species Genotypes	Strains	Distance within species
	<b>BVDV-1 (Cont'd)</b>		
<b>BVDV-1.12</b> (F, R, S)		<b>BVDV-1.12.1 (F, S):</b> 22146/81; 2561; Mousedeer; UMI136/12. <b>BVDV-1.12.2 (F, R):</b> 11207/98; 103/11; 4998/89; 51/06; 52/06; 79/11; CA/181/10; VE/245/12.	2.20
<b>BVDV-1.13 (I)</b>		<b>Deer:</b> 1; 17P; 2B; 2/VR/95; cb1; CH692; CHL1014; CHL1078; CHL1087; CHL649; CHL927; CHL928; CHL939; CHL958; CHL971; CHL972; CHL992; CHLP22; CHLP23; CHLP24; CHLP26; CHLP30; CHLP31; CHLP32; CHLP33; Iman; KS86-1ncp; M065B/93; M079B/91; M1515A/90; M245A/91; M557A/90; S-ALT5/K.	1.86
<b>BVDV-1.14 (R)</b>		TR70; TR75.	2
<b>BVDV-1.15 (M)</b>		<b>BVDV-1.15.1: ZM-95;</b> BJ10_10; BJ1020; BJ11_01; BJ1125; BJ1202; BJ1303; BJ1305; DG07; GXBH-EB20; GXBH-EB33; GXBS-LB3; GXHZ-JB24; GXLZ-BB13; GXLZ-BB5; GXLZ-BB6; GXYL-KB22; GXYL-KB53; HB-0244; HB-0273; HB-0275; HB-050030; HB-050057; HB-090913; HZ05; HZ0601; HZ0602; isolate 11; JS-01159; JS-3094; JS-03105; JS-04198; LN-1; LN317-6; LZ05; NMG311-2; NMG311-3; NMG312-26; NMG312-32; NMG313-28; NMG313-35; NMG313-55; NMG314-22; NMG314-51; NMG314-65; NMG315-1; NX0801; NX0802; NX0803; S101; S102; S12; S122; S123; S13; S21-Chima; S22; S41; S52; S62; S71; S73; S81; SD-15; SH1051; TJ0801; QHQL-216; QHQL-225; QHQL-268; XZ-141; XA08; W4-1; ZD05.	3.29
<b>BVDV-1.16 (L)</b>		<b>BVDV-1.15.2: XZ-24;</b> HB-0105; HB-050062; HB-060111; HB-080141; HB-080146; HB-080147; HB-090268; isolate 10; JS-03140; LN309-9; LN314-21; NMG313-1; NMG314-60; S72; QHHY-22; QHTJ-303887; XZ-103; XZ-109; XZ-133; XZ-176; XZ-25; XZ-48; XZ-69; XZ-71; XZ-84; XZ-86; XZ-92.	
<b>BVDV-1.17 (F)</b>		<b>TR-2007-Gu-175454-4695;</b> TR 16; TR-2007-A-2368MS; TR 72.	3
<b>BVDV-1.18 (P)</b>		<b>J;</b> AO15b@13; IT99-3690; M65CK/96; MV39CB/95; MV98CB95; R; S; W.	3
<b>BVDV-1.19 (G)</b>		<b>TJ06;</b> BJ0701; BJ0702; BJ0703; isolate 5; isolate 8.	4.20
<b>BVDV-1.20 (L, X)</b>		<b>A;</b> Bov/Ita/948.15-60; L; M657GX/95.	3.50
<b>BVDV-1.21 (U)</b>		<b>BVDV-1.20.1 (L, X): CH-01-08;</b> 71-03; 71-15; 71-16; GZ416. <b>BVDV-1.20.2 (L):</b> PG/13a/07.	5.75
<b>BVDV-1.22 (K)</b>		<b>BVDV-1.21.1:</b> 130/15-4215; 130/15-5364. <b>BVDV-1.21.2: M31182;</b> 441/09; GXBH-EB34; GXBS-LB8; GXCZ-FB7; GXCZ-FB22; GXCZ-FB25; GXHZ-JB11; GXLZ-BB4; HB-090166; JS-00108; JS-0197; JS-02007; JS-03148; JS-03198; JS-04119; JS-04138; JS-05002; JS-99054; JS-X02126; LN309-5; NMG311-20; NMG315-5; QHQL-252.	5.37
		<b>BVDV-1.22.1:</b> CH-05-b1.	6



(Cont'd)		
BVDV-1.23 (P)	BVDV-1.22.2: SuwaCp; CE2c11 (197/CA/11); Rebe; SuwaNcp. S153.	13
BVDV-2		
BVDV-2a	BVDV-2a1: variant 1: 167 237, 168 149, 173 157, 175 375, BSE921, CPA, CPAE, EFTr, HE727, Lees, MMR-T, MP, Parvo, Rubella; variant 2: 15-103, 17583-97, 23025, 37Gr, 7937, A4-124/US, AZ Spl, BSE1239, BSE341, CH515, CH649, CH809, E5-160/US, IT-1732, JZ05-1, Kosice, MAD Spl, MN Fetus, NY93, Q126, SD-06, V-FLL, WG4622, WVD829; variant 3: i4083, i61380, i628, CD87, 4-5174, Munich 1, Munich 2, 104/98, B52-2; variant 4: 11/Mi/97, 5521-95, 713-2, 97/730, BM01 isolate 11, D1-152/US, HB-1511, HLJ-10, SH-28, XJ-04, UVR420; variant 5: BD-78, C413, K1-2/CA; variant 6: 890. BVDV-2a2: variant 1.1: AF112, B45-5, B50-5, B5-4, BA/35/02, Bov/Ita/187.15-38, Bov/Ita/232.03, CplIta124.15-20, CplIta124.15-23, CplIta190.15-36, CplIta191.15-21, Ov/Ita/182.15-33, Ov/Ita188.15-18, Ov/Ita189.15-69, Ov/Ita/189.15-97, Ov/Ita/191.15-16, Ov/Ita/192.15, E1-198/CA, Giessen-1, LO/04/04, LO/08/04, LO/10/04, LO/13/04, Munich 3, BS-95-II, MS-1, OY89, PT4-02, SW90, SY-89, TC Shinozaki; variant 1.2: 17011-96; variant 2: B77-5.	1
BVDV-2b	BVDV-2a3: 301/09. BVDV-2b1: variant 1: VS-63, FI-5/BR; variant 2: VS-123-4; variant 3: 34b, ncp7; variant 4: 4p, LV56-10/13, LV/Patol02/09; variant 5: Giessen 6, LV-96, VM96, VS-260. BVDV-2b2: B5-135/MX. BVDV-2b3: Soldan. BVDV-2b4: S143, S172, S51, SD-1301.	7
BVDV-2c	098, 119, 63.	6
BVDV-2d	A3-118/US.	7
BVDV-2e	i33283.	8
BVDV-2f	BVDV-2f1: 354. BVDV-2f2: GZ546, GZ551, PT2-01, PT7-02, Ptn3, Ptn4, Ptn8.	5
BVDV-2 ND	59386, Scp	5
		7
		7
		7
		7

(Cont'd)	Species Genotypes	Strains	Distance within species
	<b>BVDV-3</b>		
	<b>BVDV-3.1</b>	BVDV-3.1.1: Variant 1: Hobi/Brazil/200/2002; D32/00_Hobi; A2/BR; Au/9SB007/09; Au/A55110-1162/09; B1/AU; B2-42/AU; B3/AU; B4-3/CA; B5-3/MX; B6/US; E4/SA; F1-29/BR; G1-35/AU; G2/BR; H1-50/AU; H3-3/US; Hobi/Brazil/315/2004; Italy-1/10-1; Italy-280/11-A; Italy-83/10-cp; Italy-83/10-ncp; IZSPLV_ToLV01/12; LV02/12; LV03/12; LV04/12; LPV-WR/BR11; PB22487; SV478/07.	1.15
		BVDV-3.1.1: Variant 2: JS12/01.	4
		BVDV-3.1.1: Variant 3: Th/04_KhonKaen.	5
		BVDV-3.1.2: HNI1507; HNI1513; HNI1518; HNI1519; HNI1524; HNI1528; HNI1537; HNI1542; HNI1559; HNI1564; HNI1568.	6.82
		BVDV-3.1.3: IndABII5385/12; IndABII5987/12; IndABII6020/12; IndABII6023/12.	7.5
		BVDV-3.1.4: BGDZS1.	8
	<b>BVDV-3.2</b>	IndBHA5296/12; IndBHA5309/12; IndBHA5426/12; IndBHA5500/12; IndBHA6060/12; IndBHA6229/12; IndBHA6332/12; IndBHA6603/12; IndBHA6604/12; IndBHA6610/12; IndMDV18963/12; IndNAR0115/12; IndNAR0116/12.	10.5
	<b>BVDV-3.3</b>	BGDZS5.	9
	<b>BVDV-3.4</b>	BGDZS3.	10
	<b>BDV</b>		
	<b>BDV-a (1)</b>	<b>BDV-a1:</b> 1062689; 135 661; 137/4; 1502304; 1505744; 170 337; A1263/2; A841/1; D1586/2; G1305; G2048; K1729/3; Q1488/1; Q1488/6; V1414; V2377/12; V2536/2.	0.91
		<b>BDV-a2:</b> 1118212; 1376527; 8320-22NZ; 8320-31NZ; 90/8320/31; 91/5809; A1870; Ch1Es; Coos Bay 5 c; Coos Bay 5 nc; FNK2012-1; FNK2012-2; FNK2012-3; FNK2012-4; JH2816; L991; Lyon2; Moredun cp; Moredun ncp; Q1673/2; T1789/1; T1802/1; V3196/1; X818.	0.93
		<b>BDV-a3:</b> BD31.	4
		<b>BDV-a4:</b> LA/5909/09; LA/8567/14, MA/14123/12.	4
	<b>BDV-b (2)</b>	BT2305; Chemnitz; Genzkow 701; J1004; LA1108; Rentier Rudolph (reindeer-1 V60-Krefeld); ST1405; ST1507; Stolpe; Wisent Casimir.	3.33
	<b>BDV-c (6, 8, Sw)</b>	<b>BDV-c1.1 (Sw):</b> CH-BD3; CH-BD4.	4
		<b>BDV-c1.2 (8):</b> Italy-103761.	3
		<b>BDV-c2 (6):</b> 06-F-0299/357; 06-F-0299/369; 06-F-0299/420; 06-F-0299/477; 10F03356; 90-F-6335; 91-F-7014; 92-F-7119; 94-F-7446/1; 94-F-7446/2; RUPI-05.	4.2

(Cont'd)		
BDV-c3 (8)	Italy-58987; Chamois-Vda-2; Chamois-Vda-3.	5
BDV-d1 (3)	AH12-01; AH12-02; AHHX15.	4.5
BDV-d2 (3)	297.	5
BDV-e1 (4)	C27.	5
BDV-e2 (4)	ZAI-1115.	6
BDV-f1 (4, 5)	0501209-052GI, 85-F-488; 89-F-5415; 93-F-7289; 96-F-7624; AV; BA/52521/09; BD/91; ESP-97; ESP97-2; ESP97-3; ESP97-4; ESP97-5.	5
BDV-f2 (4)	0502234; 2112/99; 79248/01; 80582/01; 87877/01; BDV-pig-SP-2007; M3; VFMIII.	5
BDV-f3 (4)	BU1-C4; BU-1CRA22; C121; C290; Colm24; LE31C2; Rocco.	5.57
BDV-f4 (4, 7)	AND-1; AND-2; AND-3; ARAN-1; ARAN-2; ARAN-3; ARAN-4; ARAN-5; ARAN-6; ARAN-7; ARAN-8; ARAN-9; ARAN-10; ARAN-11; ARAN-12; ARAN-13; ARAN-14; CADI-1; CADI-2; CADI-3; CADI-4; CADI-5; CADI-6; CADI-7; CADI-8; CADI-9; CADI-10; CADI-11; CADI-12; CADI-13; CERDANYA-1; CERDANYA-2; CERDANYA-3; CERDANYA-4; Chamois1; Chamois-Spain02; FRESER-2; FRESER-3; isard4606; Orlu-Etagne; Orlu-ORL 2004 02 C; Orlu-R36; Orlu-S24; Orlu-S36; PALLARS-2; PALLARS-3; PALLARS-4; PALLARS-5; PALLARS-6; PALLARS-7; RIBAGORCA-2; RIBAGORCA-3; RIBAGORCA-4; RIBAGORCA-5; sheep/272/FRA/2007.	6.66
BDV-g (TU)	BDV-g1: 10F03401; 59434/2012/EN; 70282/2007/EN; 91-F-6731; 91-F-6732; 92019/2007/AG. BDV-g2: 33S; 35; 35T; 37A; BM01 isolate 5; Lot21; RM; SNIT; SN2T; SN3G.	5.16 6.33
BDV-h (3)	JS12/04; JSLS12-01; JSYZ15.	6
BDV-i	BDV-i1: TR-13. BDV-i2: TR-14.	6 6
BDV-j (3)	BDV-j1: Ind 830-09. BDV-j2: 06-F-0083; 06M0150; 14696; 8382.1; 85-F-588; 89-F-5374; 90-F-6227; 90-F-6338; 90-F-6339; CH-BD1; LA6442110; MA783513. BDV-j3: Gifhorn; Gifhorn-sh; B300/06; CH-BD2.	4 5.85 7.33
BDV ND	BDncp; Frijters; L83/L84; R1292/01; V-TOB.	
Pestivirus I	BDV/Aydin/04-TR; BDV/Burdur/05-TR.	0

(Cont'd)	Species Genotypes	Strains	Distance within species
CSFV			
CSFV-a	CSFV-a1 (1.1; 1.2): 39; 5NCR/CSF/MZ/AIZ/348; 5NCR/CSF/MZ/AIZ/352; 1 India; 2 India; 3 India; 4 India; 5 India; 6 India; 7 India; 8 India; 9 India; 10 India; 11 India; 13 India; Alfort 187; Alfort A19; Aizawl-09; Bangalore Ind-163/07; Brescia; BRESCIAX; BV-P; C strain; CAP; cF114; CSFV212L-13; CSFV-GZ-2009; CSFV-PK15C-NG79-11; CSFV/IVRI/VB-131; CSFV/1.1/dp/CSF0382/XXXX/Koslov; CSF/MZ/KOL/73; CSF/MZ/SAI/76; CSFV/2.3/wb/CSF1046/2009/Hennef; CSFV/2.3/wb/XXXX0608/2005/Euskirchen; CSFV/2.3/Roesrath; Eystруп; GPE (-); HCLV; HCVCAD22/14; Hokkaido/66; Ibaraki/66; Ibaraki/81-115; Ibaraki/81-20; Ibaraki/81-38; Ibaraki/81-40; Ind-173/08; Ind-174/08; Ind-175/08; Ind-176/08; Ind-239/08; Ind-243/08; Ind-272/08; JL1(06); KC; LK-VNIViM (V1 GC); LOM; Miyazaki/81; Nakamura/66; NFP/AS-1; NFP/ML-2; NFP/ML-4; Rovac; RUCSFPLUM; Shimem; SWH; Vac A; Yamanashi/69.	0.54	
CSFV-a2 (2.1; 2.3): 17-93; Alfort; Chiba-80; CSFV/2.3/dp/CSF857/2006/Borken; CSFV/2.3/dp/CSF864/2007/BG/Jambuli; CSFV/2.3/dp/CSF0821/2002/HR/Novska; CSFV/2.3/wb/XXXX0609/2004/Uelzen; CSF/wb/FR5720040964-03; CSF/wb/FR672006060003-06; CSF/wb/FR6720070192-01; CSF/wb/FR6720030647-19; CSF/wb/FR6720060199-01; CSF/wb/FR6720050238-02; CSF/wb/FR6720050125-05; CSF/wb/FR6720050018-06; CSFV/2.3/SRB/1264/2005; CSFV/2.3/SRB/6168/2005; CSFV/2.3/SRB/6168/2006; GD192011; HEN03; HNLV-2011; HNSD-2012; Osaka/51; Osaka/71; Pader; Pader born; S112; S173; Shizuoka/73; SKCDK; Sp01; Switzerland 1/93; Switzerland 2/93; Switzerland 3/93/1; Switzerland 3/93/2; Switzerland 4/93; Venhorst; VRI4762; Wingene.	0.46		
CSFV-a3 (2.2): Saitama/81.		1	
CSFV-a4 (2.2): Parambi; 179/MIB/2014; 181/MIB/2014; 211/MIB/2014; 23/MIB/2014; 322/MIB/2013; 99/MIB/2014; CSFV-UP-BR-757-09.		1.4	
CSFV-a5 (2.1): IND/AS/GHY/G4.		3	
CSFV-a6 (1.3): Fukuoka/72; Honduras.		3	
CSFV-b 5440/99.		4	
CSFV-c (3) 94.4/IL/94/TWN; Kanagawa/74; Okinawa/86; Okinawa-86-2; P97.		5.5	
CSFV-d S171.		7	
CSFV ND Sch180; VRI 4167.			

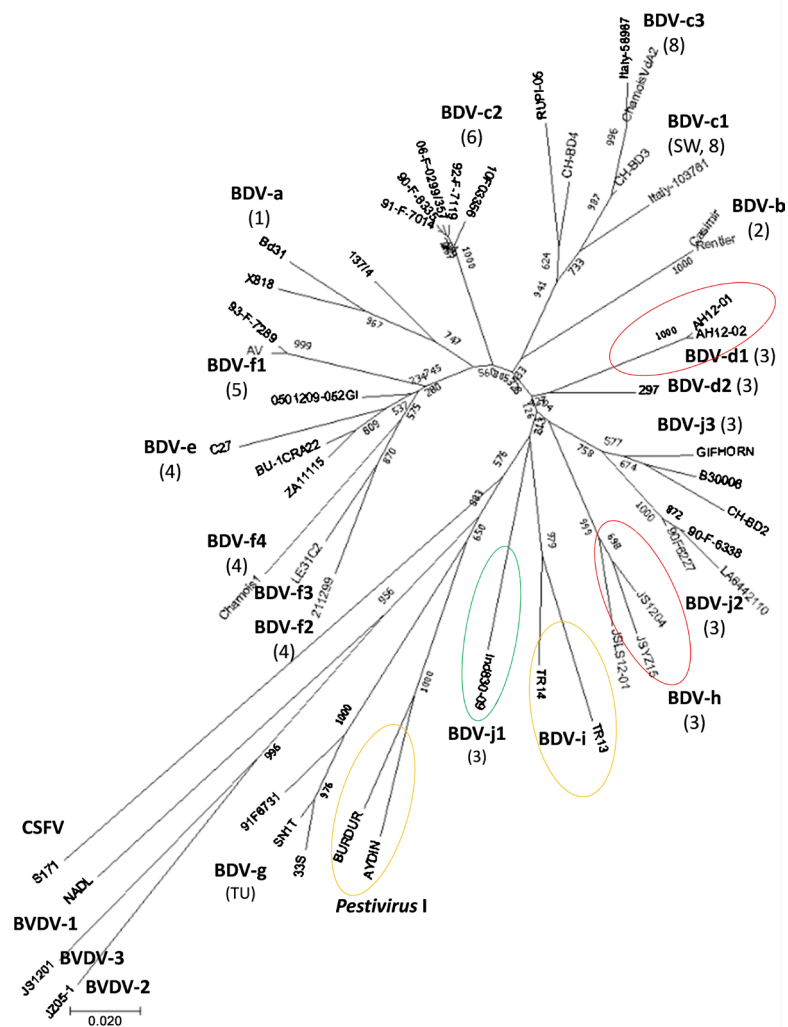
in which was characteristic, and in all the BVDV-2 species strains.

Among BVDV-1 genotypes, only BVDV-1a, 1b, 1.3 and 1.13 were widely distributed. The geographic distribution of genotypes varied considerably, and in certain countries as China, over the half of the 23 genotypes described in the species, 12 could be identified: BVDV-1a, 1b1 and 1b2, 1.3, 1.6, 1.7, 1.8, 1.10, 1.11, 1.15, 1.18, 1.21 and 1.23. BVDV-1a (n = 4) was reported in two bovines [6], two Bactrian camels [31] and one pig [32]. Despite belonging to the same BVDV-1a genotype, strains NH01 (bovine) and SH1060 (pig) were low related, showing 89.2% homology and a divergence of 11 bp in the IRES. Among BVDV-1b genotype, only two BVDV-1b1 have been reported in cattle (Zhang *et al.*, unpublished) [2], while the large majority (n = 41) were BVDV-1b2, 13 in cattle, 27 in yaks [2] and one in a Bactrian camel (*Camelus bactrianus*) [31]. Four bovine strains (Gao *et al.*, unpublished) [6] belonged to genotype BVDV-1.3. Two other bovine strains (S133 and TY05), reported as M [7] [8] resulted BVDV-1.6. One pig strain, reported as genotype O by Deng *et al.* [32] was clustered in the genotype BVDV-1.7, with four other strains all reported as genotype M, three from cattle, S43, S121 and BJ0924 [8] and one from camel, isolate 9 [31]. BVDV-1.8 (n = 5) was reported in three bovines [9] and two camels [31]. Genotype BVDV-1.10, reported as Q, resulted circulating in cattle (n = 6), pigs (n = 5) and camels (n = 3) [3] [4] [6] [8] [31] [32]. Two of the strains from camel, isolate 6 and isolate 7, have been reported as M by Gao *et al.* [31]. Only one bovine strain [8] resulted BVDV-1.11. BVDV-1.15.1 (clustered as M) were isolated in 56 bovines, 9 buffaloes, 4 Yaks, [2] [6] [7] [8] [10] 2 pigs [32] and one in a Bactrian camel [31]. BVDV-1.15.2 (28 strains) was reported as M in 13 cattle and 14 yaks [2] [8] and one in a Bactrian camel [31]. Six strains were BVDV-1.18, four cattle isolates reported as P [7] and two from Bactrian camel, isolate 5 and isolate 8, reported as M [31]. Genotype BVDV-1.21 (U) was reported in 14 bovines, 7 buffaloes and in two yaks (Sun *et al.*, unpublished) [2]. The BVDV-1.23 strain S153 [8] was isolated in cattle. In Japan most of the strains isolated from cattle and contaminant of biological products belonged to BVDV-1 genotypes 1a and 1b1. Genotypes 1.3, 1.6, 1.7, 1.8 and 1.13 were also reported.

Some genetic variants appeared to be restricted in certain areas, as certain genotypes circulating only in Turkey or China, suggesting geographic isolation [7]. Most of BVDV-1 genotypes were related to specific geographic distribution, showing two main populations circulating in Asian and European countries, respectively. Apart few exceptions, a very limited number of strains isolated in Italy belonging to 1.6, 1.8 and 1.21, some genotypes were characteristic from Asia. Genotypes 1.10, 1.15, 1.18 and 1.23 were circulating exclusively in China, and also genotype 1.21 has been reported almost exclusively in China. Other genotypes (1.6, 1.7 and 1.8) appear to be restricted to Asian or Austral Asian countries. U-A pairing in V2/2 was present only among certain Asian genotypes (1.7, 1.10 and 1.15.1). Other genotypes were rare and present only in few countries, BVDV-1.16 bovine strains reported by as genotype L [33], and strains TR70,

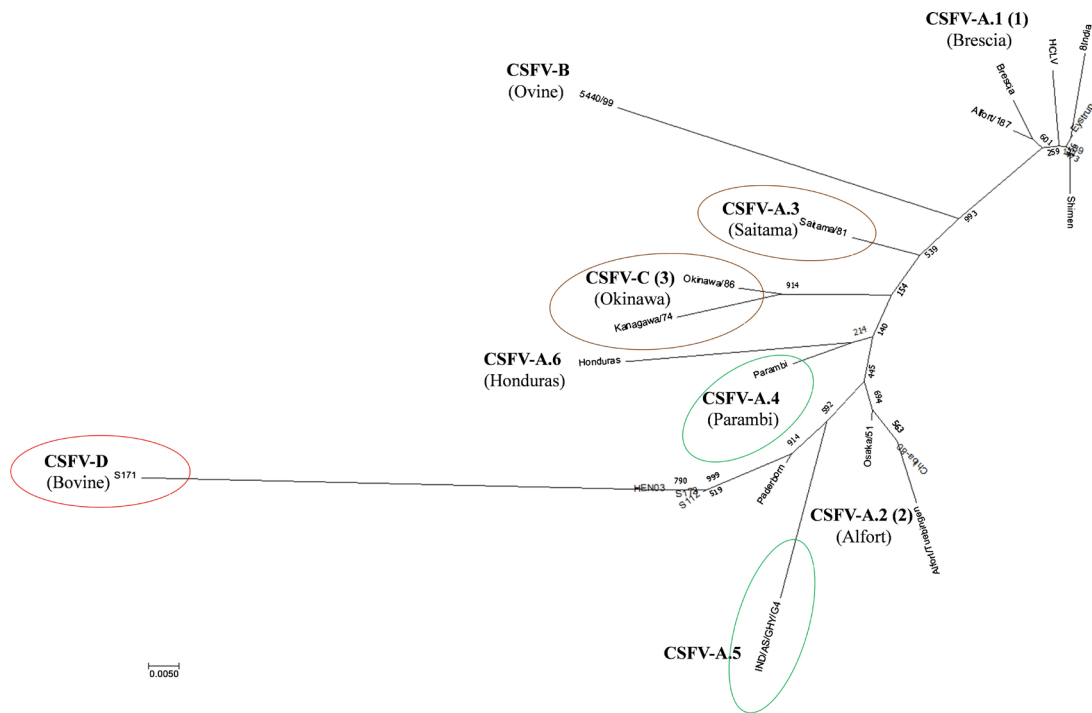




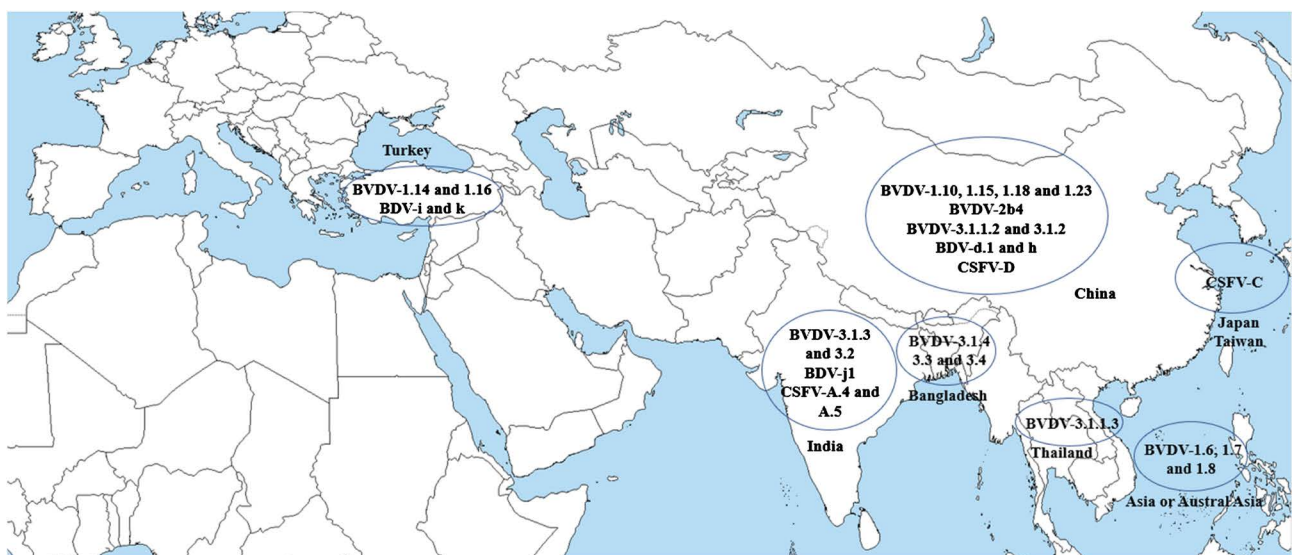


**Figure 3.** Phylogenetic tree based on the 5'-UTR comparison, suggesting a taxonomic position of the BDV strains in the genus *Pestivirus*. Strains 137/4 [U65052], X818 [AF037405], Bd31 [U70263], Casimir [AB122085] and Rentier [AB122086] are references for the genotypes BDV-a sub genotypes 1, 2 and 3 and genotype BDV-b. Strains CH-BD3 [JQ994199], CH-BD4 [JQ994200], Italy-58987 [KX573913], Italy-103761 [KT072634] and ChamoisVda2 [MG725337] are references for the genotype BDV-c sub genotypes 1 and 3 and the strains 92-F-7119 [EF693994], 92-F-7014 [EF693993], 90-F-6335 [EF693990], 06-F-0299/357 [EF694000], 10F03356 [KC859384] and RUP1-05 [KC859383] are references for the genotype BDV-c sub genotype 2. The Chinese strains AH12-01 [JQ946320] and AH12-02 [JX437132] are reference for the genotype BDV-d sub genotype 1, and strain 297 [KC484999] is reference for the BDV-d sub genotype 2. Strains C27 [DQ275623] and ZA11115 [DQ361070] are references for the BDV-e sub genotypes 1 and 2. Strains AV [EF693984], 0501209-052GI [DQ679902] and 93-F-7289 [EF693995] are references for the BDV-f sub genotype 1. Strains 2112/99 [AY159513], BU-1CRA22 [DQ275622], LE31C2 [DQ361072] and Chamois1 [AY738080] are references for the BDV-f sub genotypes 2, 3 and 4. The strain 91-F-6731 [EF988632], 33S [AF462002] and SN1T [AF461997] are reference for the genotype BDV-g (Tunisian type). The Chinese strains JS12/04 [KC537789], JSLS12-01 [KC963426] and JSYZ15 [KT327869] represent the genotype BDV-h. The Indian strain Ind830-09 [KT934377] is reference for the genotype BDV-j sub genotype 1, the strains 90-F-6338 [EF693991], 90F6227 [EF693989], LA/64421/10 [LM999989], Gifhorn [EU636997], B30006 [EU224227] and CH-BD2 [JQ994198] are reference for the genotype BDV-j sub genotypes 2 and 3. The Turkish strains TR13 [JF489888] and TR14 [JF489889] are references for the genotype BDV-i. The bovine strains NADL [M31182], the Chinese bovine strains JZ05-1 [GQ888686], JS1201 [JX469119] and S171 [KF006974] represents the BVDV-1, BVDV-2, BVDV-3 and CSFV species. The ovine Turkish strains Aydin [AM418427] and Burdur [AM418428] represents the *Pestivirus I* species. Scale bar indicates 10 nucleotide substitutions per 100 nucleotides. Genotypes present only in Asian countries are indicated in circles (red: China; green: India; orange: Turkey). Nomenclature of identified genotypes is based on divergence in the genus. Clustering according to primary structure analysis by depositors is indicated under parenthesis.





**Figure 4.** Phylogenetic tree based on the 5'-UTR comparison, suggesting a taxonomic position of the CSFV strains in the genus *Pestivirus*. Strains Brescia [M31768], 39 [AF407339], Eystrup [AF326963], Alfort/187 [X87939], HCLV [AF091507], Shimen [AF092448] and the bovine strain 8 India [MG813566] are references for the genotype CSFV-a variant 1. Strains Alfort/Tuebingen [J04358], Chiba-80 [AB019659], Osaka/51 [AB019174], Paderborn [AY072924] and the Chinese bovine strains S173 [KF006975], HEN03 [KC176778] and S112 [MK118725] are references for the genotype CSFV-a variant 2. Strain Saitama/81 [AB019178] is reference for the CSFV-a variant 3, and strains Parambi [KT239105] and Honduras [L42426] are reference for the CSFV-a variants 4 and 6. The Spanish ovine strain 5440/99 [AY159514] is reference for the genotype CSFV-b. The Japanese strains Kanagawa/74 [AB019166] and Okinawa/86 [AB019172] are references for the genotype CSFV-c. The Chinese bovine strain S171 [KF006974] represents the genotype CSFV-d. Scale bar indicates 10 nucleotide substitutions per 100 nucleotides. Genotypes present only in Asian countries are indicated in circles (red: China; green: India; brown: Japan and Taiwan). Nomenclature of identified genotypes is based on divergence in the genus. Clustering according to primary structure analysis by depositors is indicated under parenthesis.



**Figure 5.** Schematic representation of geographic distribution of Asian *Pestivirus* species genotypes circulating in restricted areas.

TR73 and TR75 belonging to the BVDV-1.14, reported by Yesilbag *et al.* [34] as genotype R, were restricted to Turkey.

In the BVDV-2 species, while BVDV-2a showed cosmopolitan diffusion and 2b and other related genotypes were more frequently distributed in South America, only one group appeared specific to Asia. The genotype b variant 4 (BVDV-2b4) included only Chinese isolates: the bovine strain SD-1301 [5] and the contaminants S143, S172 and S51 [8]. BVDV-3 species showed low heterogeneity with four different genotypes. Out of four, three (BVDV-3.2, BVDV-3.3 and BVDV-3.4) were specific to zebu and bovine isolates from India and Bangladesh, respectively [12] [13]. Strains IndMDV18963/12 and IndBHA6604/12, representative for BVDV-3.2 genotype, were divergent in the species, scoring divergence 96.66% when compared with other BVDV-3 strains. Similarly, with divergence values of 82.14% for BGD/ZS5 and 100% for BGD/ZS3, respectively, bovine strains from Bangladesh were divergent from genotypes 1 and 2, constituting a separate genotype in the BVDV-3 species. In the BVDV-3 genotype 1, subgenotype 1 variants 2 (contaminant strain JS12/01) [35] and 3 (bovine strain Th/04\_KhonKaen) [36] were reported from China and Thailand. BVDV-3 genotype 1 subgenotype 2 was specific to small ruminants from China (Shi *et al.*, unpublished), with divergence 27.27%, divergence mean value 6.82 from other BVDV-3.1 genotype strains. BVDV-3 genotype 1 subgenotypes 3 and 4 were specific to zebu and bovine isolates from India and Bangladesh, respectively [12] [13]. Strains IndABI16023/12 and IndABI16020/12, representative for BVDV-3.1.3, showed a divergence of 23.33% from strains of other genotype variants. BVDV-3.1.4 strain BGDZS1 showed a divergence of 40%.

BDV species sequence characteristics of Chinese and Turkish strains were highly divergent from other genogroups, indicating geographic segregation. Chinese strains AH12-01, AH12-02 and AHHX15, reported as genotype BDV-3 (Ghiforn type—PNS BDV-j) [37], have been clustered as genotype BDV-d, subgenotype d1. These strains showed high homology with strain 297, also reported as BDV-3 [38], clustered in the same genotype, but as separate subgenotype BDV-d2. Similarly, other Chinese strains JS12/04, JSLS12-01 and JSYZ15, reported as genotype BDV-3 [37] have been clustered as genotype BDV-h. Both genotypes resulted partially related with BDV-j. However, qualitative non correspondence at root LVP level (BDV-d GC/GU/UA-AA-AG; BDV-h GC/GU-AU/UA-AA-AU; BDV-j GC/AU/UA-UG-GC-UC) (exception AA in V3/8 of strain BDV-j2 CH-BD1, discriminated from BDV-h with additional identification marker V1/3: BDV-j2 G-C; BDV-h A-U) and quantitative evaluation of divergence values justified separate clustering. Strains of subgenotype BDV-d1 were related only with BDV-j1 (India type) (no divergence values exceeding genotype limit value 9; divergence mean value 8.5) and divergent from subgenotypes BDV-j2 (divergence 92.85%, divergence mean value 10.78) and BDV-j3 (divergence 100%, divergence mean value 11.83). Strain 297 (subgenotype BDV-d2) resulted divergent from BDV-j3 (Ghiforn type) (divergence 100%, divergence mean value

11) and related with BDV-j1 and BDV-j2 (divergence mean values 8 and 8.14, respectively), but showing lowest divergence value mean of 6.5 with BDV-d1 strains. BDV-h resulted related only with BDV-j1 (homology 33.33%; divergence mean value 8.67) and divergent from other BDV-j sub genotypes (BDV-j2: divergence 61.9%, divergence mean value 9.67; BDV-j3: divergence 100%, divergence mean value 12.33).

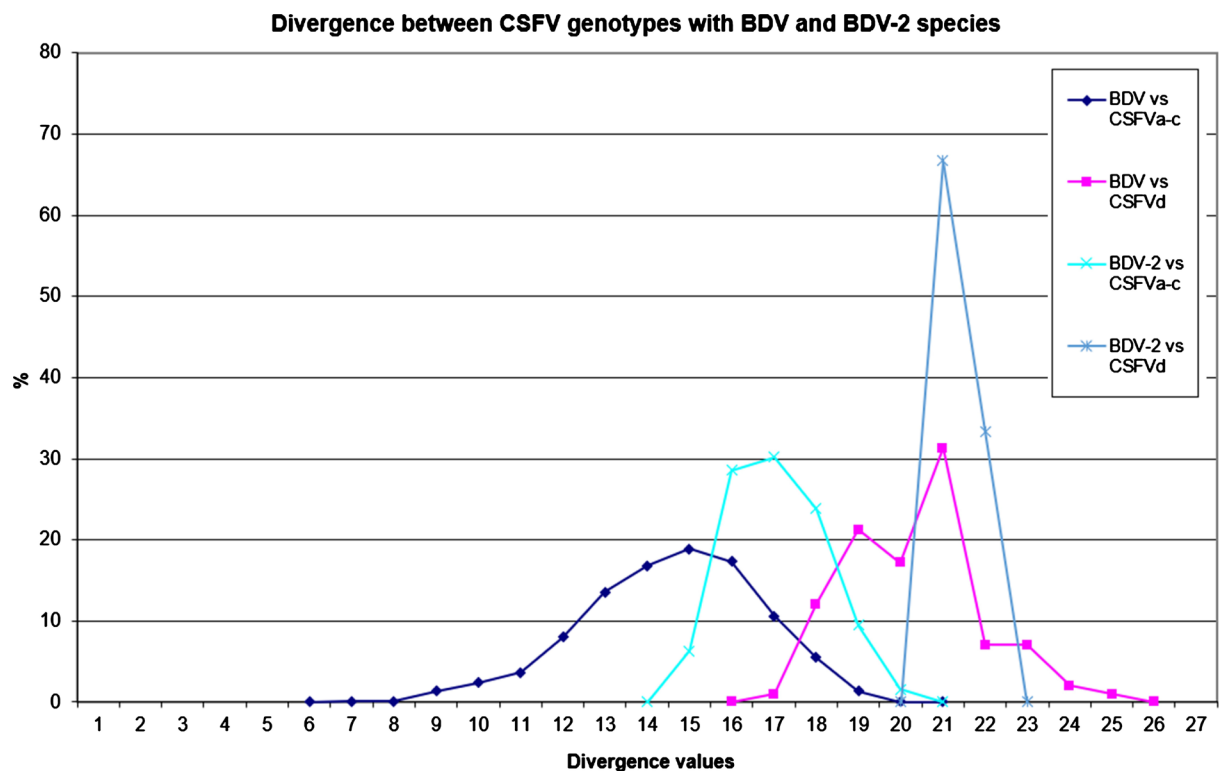
Turkish strains TR-13 and TR-14, reported as distinct group in the BDV species [39], have been clustered as genotype BDV-i. Due to the divergence value of 9, obtained comparing their respective sequences, related to nucleotide variations particularly in the V1 stem, but maintaining clear base pairing homology in the rest of the secondary structure, they have been considered as two separate sub genotypes. BDV-i1 strain TR-13 showed bulges in V1/8 C C and V1/9 G A and BDV-i2 strain TR-14 showed V1/4 G A bulge, V2/12 insertion U and V1/8 A G bulge as characteristic bp in the sequence. Comparison with the other BDV sequences, applying species determination limit value 13, resulted related to the majority of BDV genotypes, showing a divergence percentage of 36.08, with divergence value range from 7 to 19 (mean 12.91). High divergence values have been observed with genotype BDV-f (including Aveyron and Pyrenean Chamois types) (values up to 17 - 19), BDV-g (Tunisian type), sub genotype 2 (values 15 - 16). High divergence values (16-19) have been obtained also with *Pestivirus I* (Turkish type Aydin—Burdur). However, strains TR-13 and TR-14 shared all BDV species markers. Comparison with the other BDV sequences, applying genotype determination limit value 9, resulted divergent from all other BDV genotypes, despite the partial relation scored with some members of genotype BDV-a, sub genotype 2, BDV-c, sub genotype 1, and BVD-j, which, however, did not correspond to relatedness when considering the entire group to determine overall homology (out of 192 comparisons, only 19 values were 7 - 9 and 13 corresponded to the limit value). Also BDV-j1 included the Indian ovine strain Ind 830-09 [40].

In the CSFV species, secondary structure analysis revealed corresponding main genomic groups, defined by phylogenetic analysis, dividing CSFVs into three major lineages (Brescia, Alfort and Kanagawa/Okinawa types) and their sublineages [41]. Three genetic clusters referred specifically to Asian countries. The CSFV genotype a variant 4 (type Parambi) included only pig and wild boar strains from India (Ravishankar *et al.*, Tomar *et al.*, Chandramohan *et al.*, unpublished) [42]. CSFV pig strains clustered into genotype C (type Okinawa) were reported only from Japan and Taiwan (Liu, unpublished) [41] [43] [44]. The Chinese strain S171 [8], isolated from bovine serum, was clustered as CSFV-d. The strain S171 showed affinity with genotype CSFV-a2, but the difference was marked at the level of the V3 locus nucleotide base pairings. Exception made for a partial relation with CSFV-a2, S171 was divergent from all other CSFV types showing divergence values from 10 to 15. Divergence percentage with CSFV-a2 was 62.5%, with a divergence mean value of 9.87, resulting related, but belonging

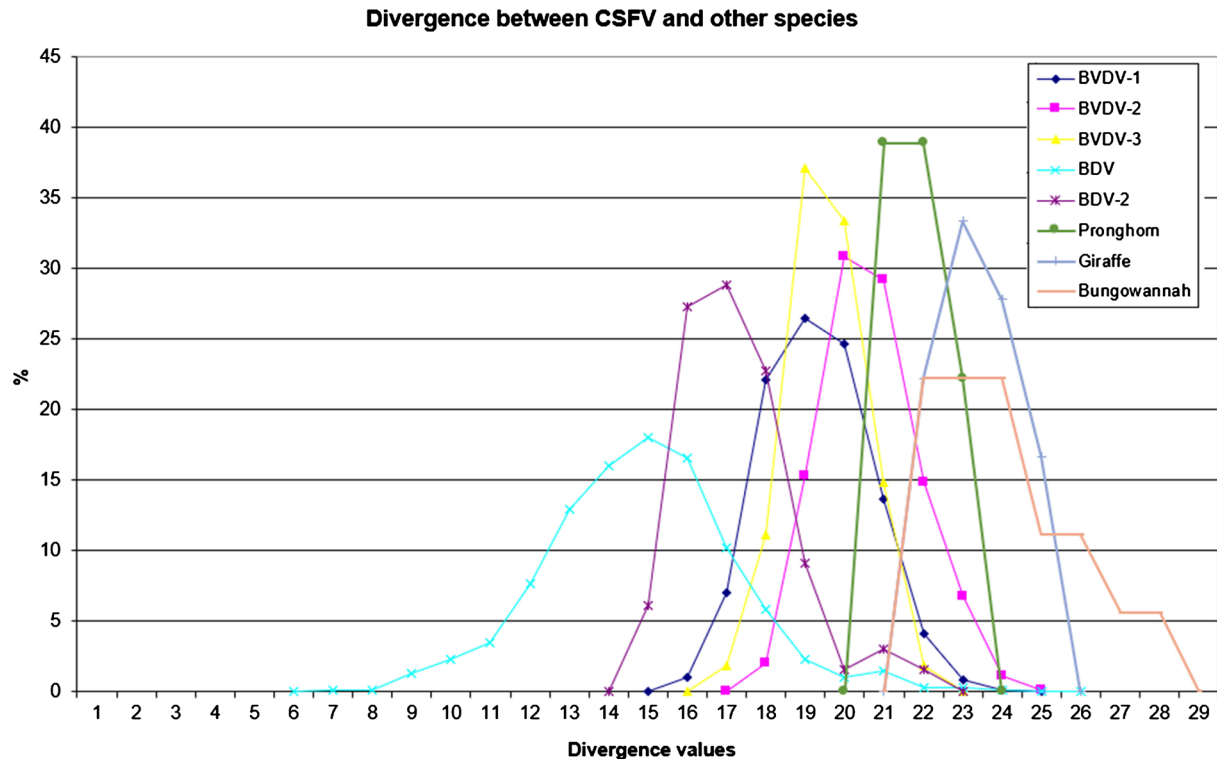
to a separate cluster. In addition, it was significantly less related to BDV species, a known characteristic of other CSFV strains (Figure 6 & Figure 7). Base pairings comparison between strain S171 and strains of the different BDV genotypes scored high divergence values, ranging from 17 to 25, with a mean value of 20.31. These values were very high in confront of the mean divergence value of 14.57 obtained comparing the other CSFV strains with BDV strains. Divergence from BDV-2 tentative species resulted also higher with CSFV-d (divergence values ranging from 21 to 22; mean divergence value 21.33), in confront of scores obtained with other CSFV genotypes CSFV-a, CSFV-b and CSFV-c (divergence values ranging from 15 to 20; mean divergence value 17.06). No relation was observed between CFSV and any other *Pestivirus* species (Figure 7). Other CSFV bovine strains have been reported from India and China, contaminants or associated to natural infection with clinical signs [8] [11] [45]. Indian strains, reported from the states of Tamil Nadu and Meghalaya, were genetically closely related to CSFV genotype a1 and a5 strains, respectively. However, a G\*U pairing in V1/3 was peculiar only in the Indian bovine strains from Tamil Nadu. In other CSFV strains this position is characterized by a conserved A-U pairing, while G-C or G\*U is conserved among BVDV-1, BVDV-2 and BVDV-3 strains (only 5 strains show exceptions, out of 1073 considered sequences).

#### 4. Discussion

Different genetic characteristics were specific to Asian clusters in different



**Figure 6.** Graphical representation of PNS quantitative evaluation. Genotype CSFV-d is not related with BDV or BDV-2.



**Figure 7.** Graphical representation of PNS quantitative evaluation. CSFV is more related with BDV, showing higher relation with BDV than with BDV-2.

*Pestivirus* species, showing great potential in the evolution of the genus. Until 1997, only two BVDV-1 genotypes were known, 1a and 1b. Thanks to the development of virologic procedures and the increased interest in the pathogen, different new genomic types have been described. However, the heterogeneity of the species become even more evident in particular by the contribution of scientists from China and other Asian countries. BVDV-1 is known to have a cosmopolitan diffusion, but genotype geographic distribution may vary considerably. For example, while predominant in UK [46], in China, BVDV-1a genotype is rare, first reported in cattle in 2010 [5] and in pigs in 2012 [32], and shows also heterogeneity, suggesting that the virus have the great variation in evolution between the different host species [5]. BVDV-1 species resulted heterogeneous especially in China. The number of different BVDV-1 genotypes increased progressively. BVDV-1b and 1m (PNS 1.15) are predominant [7]. Currently, BVDV-1 is represented by 12 types. Only in Italy, genetic diversity of BVDV-1 is higher, accounting for 14 different types [15]. Despite, the first description of the species in 1980 was related to 1b infected cattle imported from Europe [10] [47], some of the genetic variants further described appeared to be restricted to China. For example, the phylogenetic reconstructions indicated that the clustering of the Chinese BVDV-1m genotype in the phylogenetic tree is a result of geographic isolation [7]. The Chinese strain ZM-95 for many years was the only representative of a separate cluster in the species, suggesting the circulation of a rare *Pestivirus* restricted in pigs. The strain was the first BVD virus isolated in

China (Inner Mongolia) from pigs showing clinical symptoms and pathological lesions resembling mild classical swine fever [48], and characterized as genotype M [49] (PNS BVDV-1.15). Zhu *et al.* [10] reported a noncytopathic strain, genetically closely related to the pig strain ZM-95, associated with a disease characterized by high morbidity and mortality, a first BVDV-1m virus originated from cattle. In the present study, many other closely related strains have been considered from recent reports from China. Only the bovine strains BJ11\_01 and S73 isolated in China in 2011 and 2013, 16 - 18 years later (Zhang *et al.*, unpublished) [8], shared all the nucleotide characteristics of ZM-95, and all the other newly reported strains clarified genotype identification parameters. The genotype 1.15 resulted the most commonly diffused in China, and not restricted in pigs but circulating in cattle, buffaloes, yaks and Bactrian camels. The yak strain M31182 (Sun *et al.*, unpublished) showed also characteristic nucleotide base pairs indicating radical sequence variations generating new genotype in the BVDV-1 species. Reported as genotype U, PNS 1.21, the genotype was identified also in other Chinese bovine and buffalo sequences.

The heterogeneity revealed in the BDV species was among the highest observed among pestiviruses. Only BVDV-1 accounted for more genotypic variants [14]. Sequence characteristics of Turkish and Chinese strains were highly divergent from other genogroups, indicating geographic segregation. HoBi-like pestiviruses (BVDV-3) was less heterogenous, but characterized by three Asian lineages. Strains from India and Bangladesh [12] [13] showed genetic diversity and molecular characteristics clearly distinct from those previously reported circulating globally, from bovine fetal serum or naturally infected cattle and buffaloes in America, Europe, Thailand and Australia, highlighting the independent evolution of the species in the Indian subcontinent. Similarly, the CSFV species showed low heterogeneity. However, two Asian genotypes were highly divergent. According to primary sequence analysis, the strain Kanagawa/74 (PNS genotype CSFV-c) was divergent in the species [50] [51], and proposed as harmonized outgroup strain for phylogenetic analyses [52]. This suggests that strain S171 (PNS genotype CSFV-d) should be considered as another outgroup of the species with even more enhanced divergence, with atypical characteristics, possibly related to host species adaptation or a result of geographic isolation, occurrences suggested also for BVDV-1 isolates in China [5] [7]. During a survey for the detection and genetic characterization of 5'-UTR and E2 gene of CSFV from bovine population of the northeastern region of Indian state of Meghalaya, out of 134 cattle serum samples tested, all were positive in reverse transcription-polymerase chain reaction (RT-PCR) for 5'-UTR region and 10 samples were positive for CSFV antigen by a commercial antigen capture enzyme linked immunosorbent assay (Ag-ELISA) [45]. Full length E2 region of CSFV were amplified from two positive samples, CS/ML/911/IDP/13 [KY860532] and CS/ML/AF/Umiam/14 [KY860531]. Phylogenetic analysis showed similarity with isolates reported from the neighboring state of Assam, as the pig strain



IND/AS/GHY/G4 [KM362426] [53]. According to E2 region primary sequence analysis, these strains were clustered in the CSFV sub-genogroup 2.1 [45] [53]. The E2 regions of the bovine strains CS/ML/911/IDP/13 [KY860532] and CS/ML/AF/Umiyam/14 [KY860531] showed 99% nucleotide identity with IND/AS/GHY/G4 (PNS CSFV-a5) and 92% with strain Paderborn [GQ902941] (PNS CSFV-a2). 5'-UTR evaluation by PNS from complete genome of Paderborn and IND/AS/GHY/G4 suggested the appurtenance of both bovine strains CS/ML/911/IDP/13 and CS/ML/AF/Umiyam/14 to PNS CSFV-a5. As PNS CSFV-a4 (type Parambi), also this cluster was characteristic to India.

The high level of heterogeneity was reflected also in a certain confusion in the nomenclature of types. For example, the Chinese camel isolate 9, clustered as M [31], diverged of only 2 bp from the strain AQGN96BI5 of genotype 1.7 (O), and 4 bp from the 1.7 reference strain IS25CP/01 [28], thus justifying the reallocation in this BVDV group. Similarly, the Chinese camel isolate 5, reported as M [31] was reclustered in the present study, showing relation with genotype 1.17 (P) reference strain TJ06 [7] (divergence 6 bp). Confusion was also due to the use of same names to define different genetic clusters, as in the case of strains TR-2007-A-2368MS, TR-2007-Gu-175454-4695 [33], TR1, TR16 and TR72 [34], clustered as genotype L, reported from cattle in Turkey, genetically distant from homonymous isolates from continental Europe [54] [55] [56].

It is unlikely that geographic specificity of some types might be due to sampling bias, taking into account that strains have been reported from qualified laboratories, often identifying in the same epidemiological survey typical isolates. In certain cases, observations have been further validated at the level of the International Committee on Taxonomy of Viruses. For example, atypical ovine Turkish isolates BDV/Aydin/04-TR and BDV/Burdur/05-TR [57], previously considered a separate *Pestivirus* species [58], a new BDV subgroup [57] or BDV borderline candidate for reclustered as new species [59], have been recently classified as new genus *Pestivirus* member species (*Pestivirus* I) [1]. Furthermore, it is highly probable that future investigations will reveal the existence of new types in the genus since most of routinely conducted diagnostic work is based on serology and only few isolates are reported from certain regions. Similarly, despite evidence of *Pestivirus* seropositive animals in a large number of wild animal species [60], the number of reported isolates is very limited and related to few species as deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) [61] [62], or the Japanese serow (*Capricornis crispus*) [63], Pyrenean chamois (*Rupicapra pyrenaica*) [64], Alpine chamois (*Rupicapra rupicapra*) [65], and captive mousedeer (*Tragulus javanicus*) [66], wisent (*Bison bonasus*) and reindeer (*Rangifer tarandus*) [67].

Also investigations in man, may reveal new aspects of *Pestivirus* epidemiology. Using mass spectrometry proteomics to analyze protein extracts from three Zika positive brains of deceased babies with severe brain lesions and arthrogryposis, peptides from the polyprotein of a Bovine-like viral diarrhea virus have



been detected, suggesting that Zika virus may not be the only etiological agent responsible for microcephaly [68]. During the outbreak in Paraíba, in 2015, BVDV RNA was also found in the amniotic fluid collected from four mothers with babies affected by Zika and microcephaly. Two 5'-UTR RNA genomic sequences, have been provided by Prof Tanuri, Federal University of Rio de Janeiro, for further secondary structure analysis. The strain 4p, considered in the present study, belonged to genotype BVDV-2b, typical genotype circulating in South America, genetically close to Brazilian strains LV56-1013 [69] and LVPatol0209 (Silveira *et al.*, unpublished), but showing unreported variants in the IRES. The second isolate (12p) was a BVDV-1b1, genotype of cosmopolitan diffusion, very similar to bovine strains UEL9-BR/11 (Rodrigues *et al.*, unpublished), isolated in Brazil in 2011, about 10 years later than the three other closely genetically related strains 133/02, 4092/00 and 3310/01 [70] previously identified in Spain in 2002. The human strain 12p showed also homology (98% nucleotide identity) with four contaminant strains isolated in Mexico in 2012, the BVDV-1b1 NGR2, NGR3, NGR11 and NGR12 (Gomez-Romero *et al.*, unpublished). The only strain previously reported in humans belonged to genotype BVDV-1.3 [71], another cluster with cosmopolitan diffusion.

Observed heterogeneity in the *Pestivirus* species has to be considered for potential implications on diagnostic tests, control and preventive measures, since commonly available tests and vaccines are based on viral antigenic substrate [72]. Due to possible geographical segregation, recognition of the molecular characteristics of field strains present in a population is fundamental for the control or eradication programs design, vaccine development or retracing infection sources in case of outbreaks [46] [73]. Laboratory testing difficulties may be due to serological cross-reactivity or divergence among pestiviruses. Serological surveillance of BVDV by ELISA does not distinguish between BVDV and BDV as source of infection. During routine genetic typing of pestiviruses in India, BDV was detected in sheep by real time RT-PCR [40]. All the samples yielded positive virus isolates in cell culture but were found negative by a BVDV antigen ELISA, suggesting that for diagnosis of BDV infection, the commercial BVDV Ag-ELISA should be used with caution. For adequate differentiation between BVDV and BDV, cross-serum neutralization test procedure has been recently developed [74]. Current BVDV diagnostic tests may fail to detect HoBi-like viruses or to differentiate between BVDV and HoBi-like viruses [75]. Furthermore, available commercial serological tests for BVDV do not reliably detect HoBi-like virus exposure, and cross protection against HoBi-like viruses conferred by current BVDV vaccines is likely limited [75]. Therefore, accurate genetic analyses are necessary for epidemiological studies, traceability and characterization of atypical strains.

The PNS method allowed an exhaustive representation of genomic variants in the genus, based on the comparative evaluation of about 1,400 *Pestivirus* strain sequences. The particularity of the PNS method is the exclusive consideration of

strategic genomic sequences corresponding to the 5'-UTR IRES. Thus, at this level stable nucleotide variations assume high importance in terms of virus evolutionary history. Theoretically, applicable for the evaluation of all positive polarity RNA viruses, the PNS method was used for the determination of genotypes of the human hepatitis C virus [76], considering the unambiguous functional and genomic similitude between the two genera [77]. PNS sequence characterization easily discriminated species as Giraffe, Pronghorn or Bungownah [78] [79]. Even if E2 glycoprotein gene is preferred in recent taxonomy of the species [80], CSFV was evaluated with 5'-UTR, and otherwise not comparable with 5'-UTR sequences of the other different species. Despite a limiting factor for differentiation of closely related isolates [80], short length target fragments in other regions of the viral genome as 5'-UTR or NS5B have been largely applied for genotyping purposes and molecular epidemiology [50] [52] [81]. In addition, according to the Terrestrial Manual of the World Organization for Animal Health (Chapter 2.8.3 CSFV; Paragraph 1.1.5.) [82], the 5'-UTR of the CSFV genome (150 nucleotides) is also foreseen for genetic typing and molecular epidemiology of CSF. However, in light of the movement towards whole genome sequencing and recent species typing [1], the routine work performed in veterinary laboratories in the future will possibly be based on full-length genome comparison to delineate *Pestivirus* genogroups and species, despite still problematic due to lack of a sufficient number of sequences representing existing genetic variants. For example, current classification of BVDV isolates is generated using short genome fragments, giving that 97.7% of deposited sequences are restricted to 5'-UTR or Npro, mainly [16].

In addition to accurate virologic investigations, it is important to understand ways of diffusion and in particular prevent spread of atypical genetic clusters. The major concern is represented by the fact that if genetically related types induce effective cross immunity, at the contrary, divergent genetically atypical types might pose problems in terms of diagnostic reliable detection or vaccine conferred cross protection against such viruses. In addition, even non-naïve local animal populations might be more exposed in case of heterogeneous strains due to non-sufficient cross immunity. Trade of live animals is generally considered the main route of pathogen diffusion. For example, in UK, only three BVDV-1 types were detected in 1999. In 2013, phylogenetic analysis demonstrated the existence of six genotypes of BVDV-1 circulating in the country. This indicated that restocking of cattle from continental Europe has increased the genetic diversity [46]. Unless illegal animal movements occur, trade is the relatively easier controllable and preventable among ways of introduction of the virus in free herds or free areas. Contamination of biological products and iatrogenic infection are probably also relevant aspects of the virus epidemiology. This recalls also the risk of spreading exotic strains, requiring not only controls on live animals, animal products, semen, ova and embryos, but also potentially contaminated biological products. Eighty-three strains contaminant of biological

products, considered in the present study, were reported mainly from Asian countries. Commercialized batches of BVDV-1 contaminated bovine serum have been reported also in China [8]. The contamination of fetal bovine serum (FBS) as a vehicle for the rapid spread of the pathogen is a known and not really infrequent phenomenon [29] [83]. This might be the source of introduction of BVDV-3 in Italy, first identified in Europe in FBS imported from Brazil [84], and further reported in Italian cattle herds with respiratory distress and reproductive failures [85] [86] [87]. A similar hypothesis could be formulated for the BVDV-1 genotype 1.21. Some characteristic Asian strains of the genotype 1.21 have been shown to circulate also in southern Italy [88], suggesting ways of diffusion other than direct contact among animals. Being unlikely the direct import of live animals from China, other factors were implicated. The strain 441/09, isolated in 2009 in Puglia region, showed high similarity with the originally reported Chinese yak strain. The other two strains, 130/15-4215 and 130/15-5364, have been isolated six years later in 2015 in Sicily. Their sequences were very similar to each other. When compared to other BVDV-1 genotypes, both strains resulted divergent from most of other types, with high divergence values as 20 bp with the European genotype 1.22. However, the lowest divergence values were obtained with strains belonging to genotype 1.21, justifying allocation in the cluster, but in a separate sub genotype 1.21.1. These observed genomic variations were coherent with virus evolutionary adaptation related to spatial temporal factors. Strain 441/09 may represent the evidence of the introduction of an exotic *Pestivirus* variant in the animal population in Italy, while strains 130/15-4215 and 130/15-5364 may represent the result of the virus adaptational evolution in the new environment, after an adequate period of time. This suggests the introduction of genotype 1.21 from China in 2009 and the potential risk of the endemic status of the virus in the area, supported by evidence of genomic characteristics mutations, possibly related to geographical situation or contact with virus populations circulating in the region. Interestingly, the Italian bovine strain SI/207/12 [89], reported as genotype T, was clustered in the genotype 1.6, scoring low divergence values with most of the strains in the group, all reported as genotype BVDV-1n, essentially of Asian origin, including the Japanese strains so CP/75 [90] and Shitara/02/06 [91] or the South Korean strains 06z71, 06z127 (Yang and Kweon, unpublished) and KB01 (Oem *et al.*, unpublished).

Other ways of diffusion may be revealed by the unusual circulation of CSFV in animal host different from suids. CSFV is generally considered to be restricted to domestic and wild suids. Current knowledge indicates that experimental infections without clinical signs have been reported in cattle, sheep, goats and deer, but there is no evidence that these species become infected in nature [92] [93] [94] [95]. Only in one case, hog cholera virus has been identified in sheep [70]. In Spain, the reported 5440/99 ovine strain showed sequence similarities with CSFV vaccine strains used in pigs, suggesting spillovers from prophylactic campaigns. Another ovine isolate (strain 12 Ovine liver 113nt) [96], reported

from United Arab Emirates in the framework of investigations for pestiviruses, was suspected as CSFV. CSFV bovine strains detected in China might find their origin from vaccine prophylaxis performed in domestic animal populations [11]. Anti BVDV killed virus vaccines are available in China. However, for long time there were no commercial BVDV vaccines in the Chinese market. In cattle and yaks, BVDV was prevented by the wide application of triple dose of the live attenuated hog cholera lapinized vaccine (HCLV) strain in different areas of China, and especially in Tibet and Qinghai provinces. Since previous experimental studies conducted by Yuan Qingzhi in 1957 on HCLV, the only one CSFV authorized vaccine in China, efficacy and safety of the prophylactic use for BVDV in lactating and pregnant cows, calves and yaks have been demonstrated [97]. In Tibet, the immunization with HCLV of bovine species resulted beneficial in reducing losses due to BVDV infection. However, long-term use of live attenuated HCLV gave the opportunity for vaccinal strains adaptation in cattle, with further natural diffusion as possible consequence.

## 5. Conclusion

In the present study, according to secondary structure analysis, the genus *Pestivirus* resulted heterogeneous. Sequence characteristics among Asian genomic clusters within the different *Pestivirus* species suggested geographic segregation and occurrence of micro-evolutive steps in the genus evolutionary history. This aspect was particularly evident in atypical sequences originated from China or Turkey, indicating risk of diffusion by animals and products trade or contamination of biological products as bovine calf serum, with potential diagnostic and control difficulties. Determination of species heterogeneity is important for diagnostic efficiency and prophylactic purposes, taking into account possible wider animal host range by different *Pestivirus* species, causing overlapping clinical features, potential influence on eradication programs, and eventual regulatory measures.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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