Infection, Genetics and Evolution 30 (2015) 219–224

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Homologous recombination in pestiviruses: Identification of three putative novel events between different subtypes/genogroups

Matheus Nunes Weber, André Felipe Streck, Simone Silveira, Ana Cristina Sbaraini Mósena, Mariana Soares da Silva, Cláudio Wageck Canal *

Laboratório de Virologia, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 17 December 2014
Accepted 26 December 2014
Available online 3 January 2015

Keywords:
Pestivirus
Recombination
RDP
Phylogeny
Evolution

ABSTRACT

Viruses from the genus *Pestivirus* of the family *Flaviviridae* have a non-segmented, single-stranded RNA genome and can cause diseases in animals from the order Artiodactyla. Homologous recombination is rarely reported in this virus family. To detect possible recombination events, all complete pestivirus genomes that are available in GenBank were screened using distinct algorithms to detect genetic conversions and incongruent phylogenies. Three putative recombinant viruses derived from recombination from different pestivirus subtypes/genogroups were detected: *Bovine viral diarrhea virus 1* (BVDV-1) strain 3156, BVDV-2 strain JZ05-1 and *Classical swine fever virus* (CSFV) strain IND/UK/LAL-290. The present study demonstrated that the pestivirus classification cannot be based only on the analysis of one fragment of the genome because genetic conversions can lead to errors. The designation of the recombinant forms (RF) provides a more informative structure for the nomenclature of the genetic variant. The present work reinforces that homologous recombination occurs in pestivirus populations under natural replication and describes the first evidence of recombination in BVDV-2.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The viruses of the genus *Pestivirus* belong to the family *Flaviviridae*, together with the genus *Hepacivirus* (*Hepatitis C virus*, HCV) and *Flavivirus* (*Yellow fever virus*, YFV; *West Nile virus*, WNV; *Dengue virus*, DENV; and Japanese encephalitis virus, JEV). Pestiviruses have a single-stranded, positive-sense RNA genome of approximately 12.3 kilobases (kb) that contains only one open reading frame (ORF) that is flanked by non-coding regions (NCRs) at its 5′ and 3′ ends. The ORF encodes a polyprotein that is processed into twelve polypeptides: N terminal protein (N^{pro}), capsid protein (C), envelope glycoprotein (E^{rns}, E1 and E2), protein 7 (p7) and the non-structural proteins (NS) NS2, NS3, NS4A, NS4B, NS5A and NS5B (Simmonds et al., 2011).

Similar to many RNA viruses, pestiviruses exhibit high genetic heterogeneity and can be divided in four species that are recognized by the International Committee on the Taxonomy of Viruses (ICTV): *Bovine viral diarrhea virus 1* (BVDV-1), BVDV-2, *Classical swine fever virus* (CSFV) and *Border disease virus* (BDV) (Simmonds et al., 2011). These viruses also present species variants, with BVDV-1 presenting at least 17 subtypes (1a–q) (Deng et al., 2012; Vilcek et al., 2001), BVDV-2 presenting three subtypes (2a–c) (Flores et al., 2002; Jenckel et al., 2014), BDV presenting seven genotypes (Becher et al., 2003; Giammarioli et al., 2011), and CSFV presenting three genogroups (1, 2 and 3) that can each be divided into three or four subgenogroups (Greiser-Wilke et al., 1998; Lowings et al., 1996; Pan et al., 2005). In addition to the established species, new putative viruses with significant genetic and antigenic differences were already detected, i.e., the pestivirus of giraffe (Avalos-Ramirez et al., 2001), 'HoBi'-like viruses (Schirrmeier et al., 2004), Pronghorn virus (Vilcek et al., 2005), Bungowannah virus (Kirkland et al., 2007) and two other viruses that were detected in small ruminants from Tunisia (Thabti et al., 2005) and Turkey (Oguzoglu et al., 2009).

Heterologous recombination (or non-homologous recombination) in pestiviruses was already reported to generate the cytopathic (cp) biotype that can evolve from non-cytopathic (ncp) viruses and cellular sequences (Becher and Tautz, 2011; Hughes, 2004).

Instead, homologous recombination is an important evolutionary process for many viruses (Simon-Loriere and Holmes, 2011) and was previously identified in *Flaviviridae* members such as DENV (Chen et al., 2008; Tolou et al., 2001; Villabona-Arenas et al., 2013) and HCV (Kalinina et al., 2002; Shi et al., 2012), as well as BVDV-1 (Jones and Weber, 2004) and CSFV (He et al., 2007) from

* Corresponding author. Tel.: +55 51 33086926; fax: +55 51 33087305.
E-mail address: claudio.canal@ufrgs.br (C.W. Canal).

http://dx.doi.org/10.1016/j.meegid.2014.12.032
1567-1348/© 2015 Elsevier B.V. All rights reserved.

the pestivirus genus. Previous studies demonstrated that virus classification cannot be based only on the analysis of one fragment of the genome since genetic conversions can lead to errors and proposed the designation of the recombinant forms (Kalinina et al., 2002; Jones and Weber, 2004). Thus, the goal of the present study was to search, identify and determine the distribution of putative recombination events using several algorithms to detect genetic conversions in all complete pestivirus genomes that are available in GenBank.

2. Materials and methods

The available whole-genome sequences of 125 pestiviruses were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) on July 13th, 2014 (Table A.1). The genome sequences were from strains of recognized species and their subdivisions as well as atypical species. The dataset was aligned using ClustalW and the BioEdit version 7.2.5 software (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

Putative recombination events were verified using the Recombination Detection Program version 4 (RDP4) software (http://web.cbio.uct.ac.za/~darren/rdp.html) with the default settings. The software used several algorithms, including RDP (Martin and Rybicki, 2000), GENECONV (Padidam et al., 1999), BootScan (Martin et al., 2005), MaxChi (Smith, 1992), Chimaera (Posada and Crandall, 2001), SiScan (Gibbs et al., 2000) and 3Seq (Boni et al., 2007). The beginning and end breakpoints of the potential recombinant sequences were also defined by the RDP4 software. Putative recombinant events were considered significant when $P \leqslant 0.01$ was observed for the same event using four or more algorithms. The already-described, recombinant pestivirus BVDV-1 strain ILLNC (GenBank accession number U86600.1) (Jones and Weber, 2004), CSFV strain 39 (GenBank accession number AF407339.1) (He et al., 2007) and CSFV strains ALD (GenBank accession number D49532.1) and SWH (GenBank accession number DQ127910.1) (Ji et al., 2014) were used to analyze the performance of the applied methodology.

Phylogenetic analysis was performed to visualize possible relationships between the putative recombinant strains and other pestiviruses. The beginning and end breakpoints of the potential recombinant strains were used to define the cutoff and to segregate the genomes into three or four segments to perform independent analyses. Molecular Evolutionary Genetics Analysis version 6 (MEGA6) (Tamura et al., 2013) was used for phylogeny inference according to the maximum likelihood algorithm. The nucleotide substitution model was defined by the tool "find best DNA/Protein model (ML)" of MEGA6. The robustness of the hypothesis was tested with 1000 non-parametric bootstrap analyses.

All the sequence alignments used to perform all the analysis for RDP4 and to construct the phylogenetic trees are available in Figshare (http://figshare.com/) with the DOI number http://dx.doi.org/10.6084/m9.figshare.1272825.

3. Results

Three novel putative events were found with *P* values lower than 0.01 (Table 1). The results showed that three pestivirus strains (3156/BVDV-1, JZ05-1/BVDV-2 and IND/UK/LAL-290/CSFV) are potential recombinant viruses derived from parental viruses with different subtypes/genogroups. The possible major and minor parents of the putative recombinants as well as the beginning and end breakpoints were also defined by RDP4 and are shown in Table 1 and Fig. 1. The BVDV-1 strain ILLNC and the CSFV strains 39, ALD and SWH which were analyzed as controls of known recombination events, confirmed the previous findings (He et al., 2007; Ji et al., 2014; Jones and Weber, 2004).

The putative BVDV-1 recombinant strain 3156 (GenBank accession number JN704144.1) had the SD1 (BVDV-1a) strain (GenBank accession number M96751.1) as its major parent and GX4 (BVDV-1b) as its minor parent (GenBank accession number KJ689448.1). In the putative recombination event, the SD1 sequence had replacements of two homologous regions derived from GX4: the first one ending in the glycoprotein E2 gene and the second had its beginning and end breakpoints between the NS2 and NS5A genes (Fig. 1A).

The putative recombinant strain JZ05-1 (GenBank accession number GQ888686.2) had the 11F011 (BVDV-2a) strain (GenBank accession number KC963968.1) as its major parent. However, the RDP4 software could not find the minor parent in the sequences available in Genbank and defined it as unknown. The putative region of recombination between 11F011 and the unknown parent strain was located between the p7 and NS4B genes (Fig. 1B). The fragment of the genome of JZ05-1 that was obtained from the unknown origin (located between the breakpoints) was also submitted to a MegaBlast search (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) to find a possible minor parent, and the result showed only 89.5% identity with BVDV-2b strain SD1301 (GenBank accession number KJ000672.1).

The putative recombinant strain IND/UK/LAL-290 (GenBank accession number KC851953.1) had the Bergen strain CSFV-2.2 (GenBank accession number KJ619377.1) as its major parent. The RDP4 software could also not define the minor parent in the sequences available in GenBank. The putative recombination region between Bergen and this unknown parent strain was located between the NS3 and the NS5A genes (Fig. 1C). The

Table 1
Putative recombination events in pestiviruses detected using the RDP4 software.

Recombinant		3156 (JN704144.1)	3156 (JN704144.1)	JZ05-1 (GQ888686.2)	IND/UK/LAL-290 (KC851953.1)
Major parent		SD1 (M96751.1)	SD1 (M96751.1)	11F011 (KC963968.1)	Bergen (KJ619377.1)
Minor parent		GX4 (KJ689448.1)	GX4 (KJ689448.1)	Unknown strain	Unknown strain
P-values determined by seven different programs	RDP	5.085×10^{-201}	9.299×10^{-154}	7.195×10^{-17}	7.902×10^{-7}
	GENECONV	4.770×10^{-192}	1.924×10^{-135}	ND	7.093×10^{-3}
	BootScan	1.330×10^{-199}	8.486×10^{-152}	6.248×10^{-16}	1.896×10^{-4}
	MaxChi	7.433×10^{-54}	5.133×10^{-57}	4.824×10^{-16}	1.355×10^{-7}
	Chimaera	8.489×10^{-58}	2.318×10^{-57}	5.358×10^{-15}	1.223×10^{-6}
	Siscan	2.985×10^{-55}	3.079×10^{-78}	1.353×10^{-23}	5.966×10^{-6}
	3Seq	2.684×10^{-303}	6.752×10^{-170}	7.320×10^{-10}	5.445×10^{-4}
Beginning breakpoint (position in alignment)		–	4469 (99% CI: 4436–4490)	3637 (99% CI: 3622–3694)	5995 (99% CI: 5880–6145)
End breakpoint (position in alignment)		2871 (99% CI: 2852–2874)	8715 (99% CI: 8698–8718)	7360 (99% CI: 7342–7372)	7296 (99% CI: 7224–7363)

ND: Recombination not detected with this algorithm.

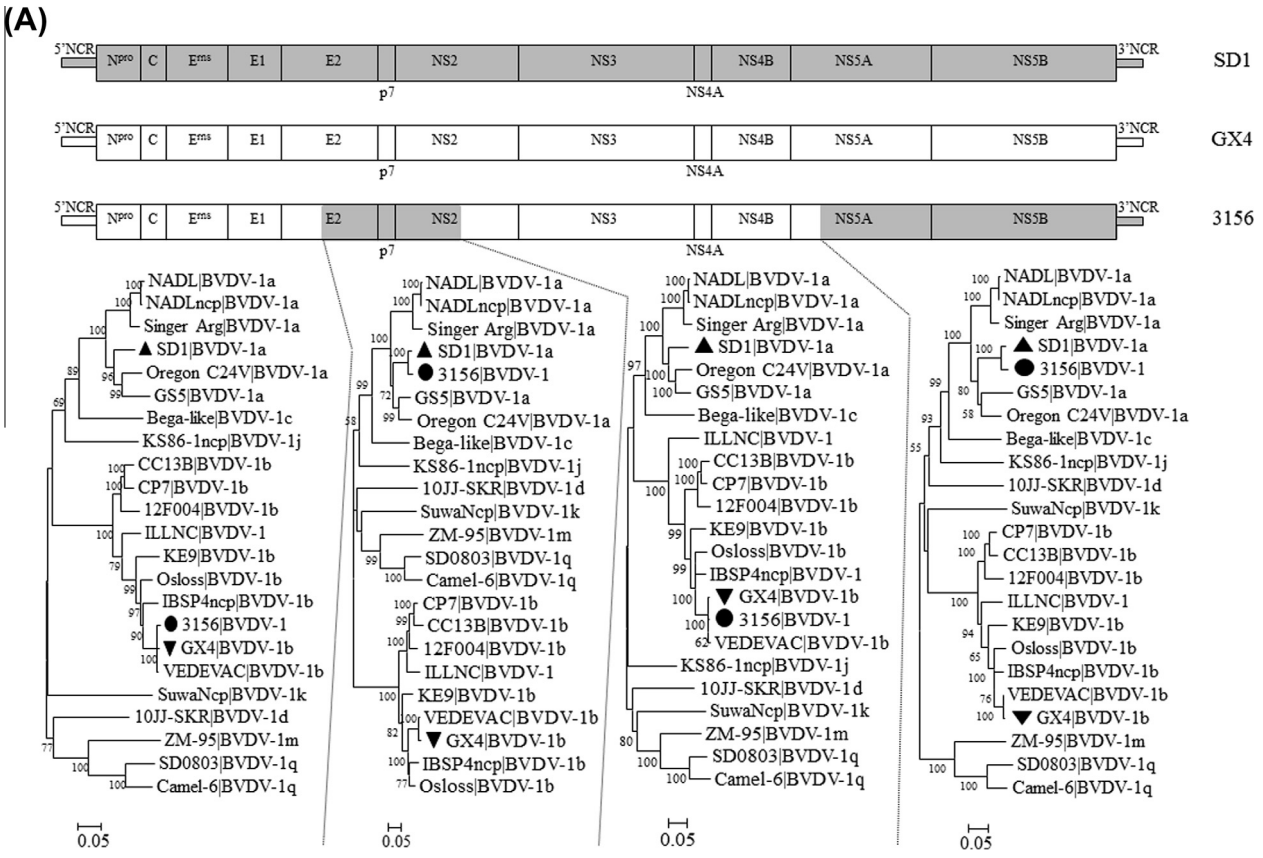

Fig. 1. Schematic representation of the potential recombination events in pestiviruses. The genome organization of the potential recombinant viruses, with the breakpoints and segments of the genome derived from the major and minor parents, are schematically represented. The relationship between the putative recombinant (●) and major (▲) and minor (▼) parents in the different segments of the genome is shown in the maximum likelihood rectangular trees, where bootstrap values ⩾50% are represented for strains 3156 (A), JZ05-1 (B) and IND/UK/LAL-290 (C).

fragment of the genome of IND/UK/LAL-290 with unknown origin (located between the breakpoints) was also submitted to a Mega-Blast search to find a possible minor parent, and this analysis resulted in only 84% identity with the recombinant CSFV strain 39 (GenBank accession number AF407339.1).

To visualize the incongruences in the phylogenetic analysis, ten different estimations from different genomic regions between potential recombinant strains and their putative major and minor parents were constructed. The nucleotide substitution model selected by MEGA6 was General Time Reversible with gamma distributed with invariant sites (GTR + G + I) for all the analysis with exception of the third segment of the BVDV-2 analysis where GTR + G was selected.

The analysis of strain 3156 (BVDV-1) showed that this strain clustered with BVDV-1a strains in two trees (Fig. 1A). In the other two trees, the putative recombinant clustered with BVDV-1b strains. All of these nodes were supported by 100% bootstrap values.

The analysis of strain JZ05-1 (BVDV-2) showed that, in trees constructed using the downstream region of the beginning breakpoint and upstream region of the end breakpoint, this strain clustered in a branch formed by its major parent (11F011) and other BVDV-2a strains (890, C413, p11Q and New York93) (Fig. 1B). These branches were also supported by 100% bootstrap values. However, in the tree constructed with the segment of the genome between the beginning and the end breakpoints, JZ05-1 clustered in the same terminal node as BVDV-2b strains Hokudai-Lab/09 (GenBank accession number: AB567658.1) and SD1301 (GenBank accession number: KJ000672.1), and this result was supported by a 90% bootstrap value. This information led to the conclusion that the other non-recombinant parent is the ancestor of the clade formed by Hokudai-Lab/09 and SD1301.

For CSFV, strain IND/UK/LAL-290 clustered in the branch of CSFV-2.2 (90% bootstrap value) and in the same terminal node as CSFV strain 39, and this result was supported by a bootstrap value of 100%. In the analysis of the region between the breakpoints, strain IND/UK/LAL-290 clustered in a unique branch with strain 39, and this result was supported by a 100% bootstrap value. In the tree constructed with the segment upstream the end breakpoint, IND/UK/LAL-290 clustered in the same branch of the Bergen strain, and this result was supported by a 100% bootstrap value (Fig. 1C). The location of strain 39, however, changed.

4. Discussion

Natural recombination has been described in members of *Flaviviridae*, i.e., *Flavivirus* and *Hepacivirus* (Chen et al., 2008; Kalinina et al., 2002; Shi et al., 2012; Tolou et al., 2001; Villabona-Arenas et al., 2013); however, there are only four reports of natural recombination for the genus *Pestivirus*, i.e., one in BVDV-1 and three for CSFV (He et al., 2007; Ji et al., 2014; Jones and Weber, 2004). In the present report, strong evidence for the occurrence of homologous recombination in BVDV-1 and BVDV-2 from different subtype strains and of CSFV from the same or different genogroups was supported by at least six of the seven algorithms that were used to detect genetic conversion (Table 1) and by the phylogenetic analysis. The data reveals that strain 3156, a BVDV-1 detected in cattle from China in 2011, is a putative recombinant between BVDV-1a and BVDV-1b (Fig. 1). A recombination between BVDV-1a and 1b

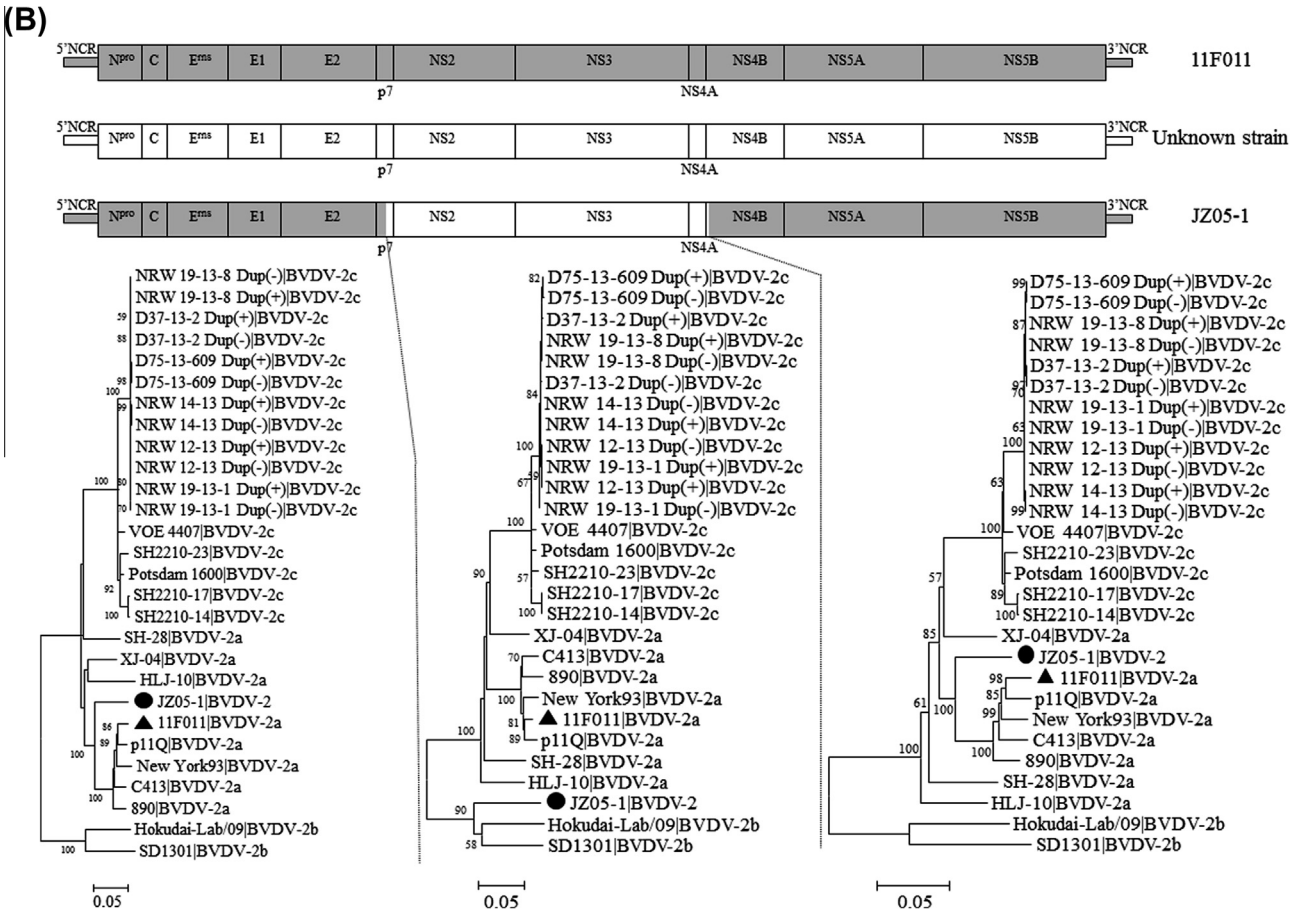

Fig. 1 (*continued*)

was previously reported (Jones and Weber, 2004), and the data reported in the present study reinforces that this phenomena can take place in natural viral populations as an important evolutionary event. Furthermore, it is important to highlight that strain 3156 is deposited in GenBank as a BVDV-1b strain, although this virus can be classified as BVDV-1a or 1b according to the genomic region that is analyzed.

The present data also reveals that strain JZ05-1, a cp virus detected in cattle from China in 2005, is a possible recombinant between the BVDV-2a and BVDV-2b strains (Fig. 1B). However, it can be observed in the maximum likelihood trees that an ancient recombination (strain JZ05-1) also occurred where the exact parental strains cannot be pinpointed, and also a case where it cannot be certain about which are the parental and which are the recombinants. This hypothesis can be better visualized in the phylogenetic trees since JZ05-1 is not located in the same terminal node as the sequences classified as major and minor parents, but apparently emerged in independent terminal nodes in all the trees. This is the first evidence of recombination for BVDV-2.

For CSFV, homologous recombination from different genogroups has been reported (He et al., 2007). The present findings point that strain IND/UK/LAL-290, a CSFV detected in a backyard pig from India, generated by a homologous recombination between a CSFV-2.2 and a CSFV other than from genogroup 2 (Fig. 1C). In two of the maximum likelihood trees it can be observed that IND/UK/LAL-290 is located in the CSFV-2 branch closely related to strain Bergen and in the third one in a branch that has the same ancestor of the CSFV-2 genogroup. Moreover, it evolved independently creating a branch where IND/UK/LAL-290 is closely related to strain 39, another putative recombinant strain (He et al., 2007). It can be deduced that an ancestor of strain 39 participated in the generation of strain IND/UK/LAL-290. Furthermore, this virus was classified as CSFV-2.2 (Kumar et al., 2014), despite the incongruences that could be observed in its phylogeny.

It is important to reinforce that RDP4 define the parental sequences based on pairwise comparisons between the query (putative recombinant) and reference sequences used in the analysis. This means that the putative recombinant and the possible parental sequences are both descendants of an acestor that was possibly better represented by the common node at the phylogeny.

Potential recombination events between different pestivirus species were not found. Interspecies recombination in pestiviruses can be an extremely rare process because gene transfer between different genomes require physical cohabitation of both genomes in the same cell (Simon-Loriere and Holmes, 2011) and viremia lasts at least 10 days and results in a sterile immunity (MacLachlan and Dubovi, 2011). Furthermore, to be able to spread further, the recombinant virus must not only be viable but also have to compete with both parental strains (Simon-Loriere and Holmes, 2011). It is important to reinforce that the homologous recombinations observed in the present work may have emerged artificially, intentionally or not since the study is based on data from a public database and all these putative recombinants are single rather than a group.

The BVDV-1, BVDV-2, CSFV and BDV are classified into variants within their respective species (Becher et al., 2003; Flores et al., 2002; Lowings et al., 1996; Vilcek et al., 2001), but recombinant strains carry incongruences in these classifications because of their evolutionary origin. The present study demonstrated that the pestivirus classification cannot be based only on the analysis of one fragment of the genome because genetic conversions can lead to errors. For example, the designation of the recombinant forms

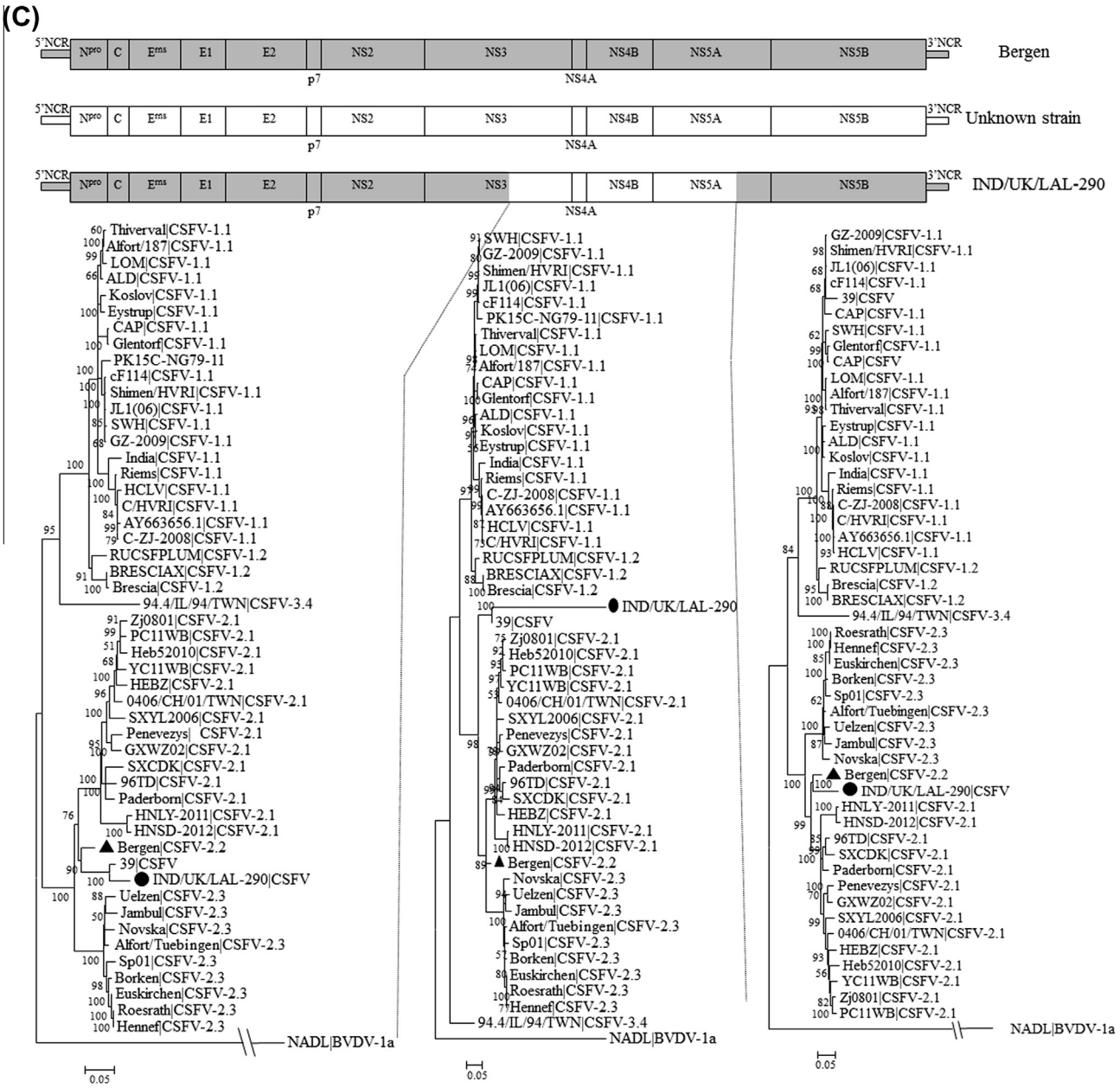

Fig. 1 (*continued*)

(RF), following the proposal of Jones and Weber (2004) for BVDV-1 and of Kalinina et al. (2002) for HCV, provides a more informative structure for the nomenclature of the genetic variant. According to this nomenclature, we propose the designation: RF1a1b and RF2a2b for viruses exhibiting the inter-subtype recombination in BVDV-1 and BVDV-2, respectively. The nomenclature for the CSFV from different genogroups reported herein is difficult to establish because the genogroup of the minor parent was not identified, and therefore, it was not possible to define it in the phylogeny.

Because the present study found that 5.6% (7 of 125) of the full-length analyzed genomes are potential recombinant viruses, homologous recombination in pestiviruses is more frequent than what has been reported for the other *Flaviviridae* members (Shi et al., 2012). Furthermore, as could be observed for HCV and DENV (Chen et al., 2008; Kalinina et al., 2002; Shi et al., 2012; Tolou et al., 2001), the location of the homologous recombination in the genome of the potential recombinant pestiviruses was random.

In the present study, three putative novel recombination events in pestiviruses were detected, showing the first evidence of recombination in BVDV-2 and reinforcing the role of these horizontal gene transfer events in the evolution of BVDV-1 and CSFV. For pestiviruses, these events are apparently even more frequent than in other *Flaviviridae* members. Moreover, the existence of recombinant strains can represent a challenge for phylogenetic and taxonomic studies. The evolutionary consequences of viral homologous recombination must be further understood to determine the extent to which recombination plays a role in pestivirus evolution and to establish adequate theoretical frames for the study of viral phylogenies.

Acknowledgments

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do

Sul (FAPERGS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Propesq/UFRGS supported this study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2014.12.032.

References

Avalos-Ramirez, R., Orlich, M., Thiel, H.J., Becher, P., 2001. Evidence for the presence of two novel pestivirus species. Virology 286, 456–465.

Becher, P., Tautz, N., 2011. RNA recombination in pestiviruses: cellular RNA sequences in viral genomes highlight the role of host factors for viral persistence and lethal disease. RNA Biol. 8, 216–224.

Becher, P., Avalos Ramirez, R., Orlich, M., Cedillo Rosales, S., König, M., Schweizer, M., Stalder, H., Schirrmeier, H., Thiel, H.-J., 2003. Genetic and antigenic characterization of novel pestivirus genotypes: implications for classification. Virology 311, 96–104.

Boni, M.F., Posada, D., Feldman, M.W., 2007. An exact nonparametric method for inferring mosaic structure in sequence triplets. Genetics 1047, 1035–1047.

Chen, S.P., Yu, M., Jiang, T., Deng, Y.Q., Qin, C.F., Han, J.F., Qin, E.D., 2008. Identification of a recombinant dengue virus type 1 with 3 recombination regions in natural populations in Guangdong province, China. Arch. Virol. 153, 1175–1179.

Deng, Y., Sun, C.-Q., Cao, S.-J., Lin, T., Yuan, S.-S., Zhang, H.-B., Zhai, S.-L., Huang, L., Shan, T.-L., Zheng, H., Wen, X.-T., Tong, G.-Z., 2012. High prevalence of bovine viral diarrhea virus 1 in Chinese swine herds. Vet. Microbiol. 159, 490–493.

Flores, E.F., Ridpath, J.F., Weiblen, R., Vogel, F.S.F., Gil, L.H.V.G., 2002. Phylogenetic analysis of Brazilian bovine viral diarrhea virus type 2 (BVDV-2) isolates: evidence for a subgenotype within BVDV-2. Virus Res. 87, 51–60.

Giammarioli, M., La Rocca, S.A., Steinbach, F., Casciari, C., De Mia, G.M., 2011. Genetic and antigenic typing of border disease virus (BDV) isolates from Italy reveals the existence of a novel BDV group. Vet. Microbiol. 147, 231–236.

Gibbs, M.J., Armstrong, J.S., Gibbs, A.J., 2000. Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. Bioinformatics 16, 573–582.

Greiser-Wilke, I., Depner, K., Fritzemeier, J., Haas, L., Moening, V., 1998. Application of a computer program for genetic typing of classical swine fever virus isolates from Germany. J. Virol. Methods 75, 141–150.

He, C.Q., Ding, N.Z., Chen, J.G., Li, Y.L., 2007. Evidence of natural recombination in classical swine fever virus. Virus Res. 126, 179–185.

Hughes, A.L., 2004. Evolutionary origin of the Jiv90 gene of pestivirus. Infect. Genet. Evol. 4, 329–333.

Jenckel, M., Höper, D., Schirrmeier, H., Goller, K.V., Hoffmann, B., Beer, M., 2014. Mixed triple: allied viruses in unique recent isolates of highly virulent type 2 bovine viral diarrhea virus detected by deep sequencing. J. Virol. Methods 88, 6983–6992.

Ji, W., Niu, D.D., Si, H.L., Ding, N.Z., He, C.Q., 2014. Vaccination influences the evolution of classical swine fever virus. Infect. Genet. Evol. 25, 69–77.

Jones, L.R., Weber, E.L., 2004. Homologous recombination in bovine pestiviruses phylogenetic and statistic evidence. Infect. Genet. Evol. 4, 335–343.

Kalinina, O., Norder, H., Mukomolov, S., Magnius, L.O., 2002. A natural intergenotypic recombinant of hepatitis C virus identified in St. Petersburg. J. Virol. 76, 4034–4043.

Kirkland, P.D., Frost, M.J., Finlaison, D.S., King, K.R., Ridpath, J.F., Gu, X., 2007. Identification of a novel virus in pigs – Bungowannah virus: a possible new species of pestivirus. Virus Res. 129, 26–34.

Kumar, R., Rajak, K., Chandra, T., Thapliyal, A., Muthuchelvan, D., Sudhakar, B., 2014. Whole-genome sequence of a classical swine fever virus isolated from the Uttarakhand state of India. Genome Announc. 2, e00371–14.

Lowings, P., Ibata, G., Needham, J., Paton, D., 1996. Classical swine fever virus diversity and evolution. J. Gen. Virol. 77, 1311–1321.

MacLachlan, N.J., Dubovi, E.J., 2011. Flaviviridae. In: MacLachlan, N.J., Dubovi, E.J. (Eds.), Fenner's Veterinary Virology. Academic Press, London, pp. 467–481.

Martin, D., Rybicki, E., 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics 16, 562–563.

Martin, D.P., Posada, D., Crandall, K.A., Williamson, C., 2005. A modified bootscan algorithm for automated identification of recombinant sequences and recombination breakpoints. AIDS Res. Hum. Retroviruses 21, 98–102.

Oguzoglu, T.C., Tan, M.T., Toplu, N., Demir, A.B., Bilge-Dagalp, S., Karaoglu, T., Ozkul, A., Alkan, F., Burgu, I., Haas, L., Greiser-Wilke, I., 2009. Border disease virus (BDV) infections of small ruminants in Turkey: a new BDV subgroup? Vet. Microbiol.

Padidam, M., Sawyer, S., Fauquet, C.M., 1999. Possible emergence of new geminiviruses by frequent recombination. Virology 265, 218–225.

Pan, C.H., Jong, M.H., Huang, T.S., Liu, H.F., Lin, S.Y., Lai, S.S., 2005. Phylogenetic analysis of classical swine fever virus in Taiwan. Arch. Virol. 150, 1101–1119.

Posada, D., Crandall, K.A., 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. PNAS 98, 13757–13762.

Schirrmeier, H., Strebelow, G., Depner, K., Hoffmann, B., Beer, M., 2004. Genetic and antigenic characterization of an atypical pestivirus isolate, a putative member of a novel pestivirus species. J. Gen. Virol. 85, 3647–3652.

Shi, W., Freitas, I.T., Zhu, C., Zheng, W., Hall, W.W., Higgins, D.G., 2012. Recombination in hepatitis C virus: identification of four novel naturally occurring inter-subtype recombinants. PLoS ONE 7, e41997.

Simmonds, P., Becher, P., Collet, M.S., Gould, E.A., Heinz, F.X., Meyers, G., Monath, T., Pletnev, A., Rice, C.M., Stiansny, K., Thiel, H.J., Weiner, A., Bukhet, J., 2011. Flaviviridae. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, pp. 1003–1020.

Simon-Loriere, E., Holmes, E.C., 2011. Why do RNA viruses recombine? Nat. Rev. Microbiol. 9, 617–626.

Smith, J.M., 1992. Analyzing the mosaic structure of genes. J. Mol. Evol. 34, 126–129.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Thabti, F., Letellier, C., Hammami, S., Pépin, M., Ribière, M., Mesplède, A., Kerkhofs, P., Russo, P., 2005. Detection of a novel border disease virus subgroup in Tunisian sheep. Arch. Virol. 150, 215–229.

Tolou, H.J.G., Couissinier-Paris, P., Durand, J.P., Mercier, V., Pina, J.J., Micco, P., Billoir, F., Charrel, R.N., Lamballerie, X., 2001. Evidence for recombination in natural populations of dengue virus type 1 based on the analysis of complete genome sequences. J. Gen. Virol. 82, 1283–1290.

Vilcek, S., Paton, D.J., Durkovic, B., Strojny, L., Ibata, G., Moussa, A., Loitsch, A., Rossmanith, W., Vega, S., Scicluna, M.T., Paifi, V., 2001. Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. Arch. Virol. 146, 99–115.

Vilcek, S., Ridpath, J.F., Van Campen, H., Cavender, J.L., Warg, J., 2005. Characterization of a novel pestivirus originating from a pronghorn antelope. Virus Res. 108, 187–193.

Villabona-Arenas, C.J., Brito, A.F., Zanotto, P.M.A., 2013. Genomic mosaicism in two strains of dengue virus type 3. Infect. Genet. Evol. 18, 202–212.