27. Sun, Q., Guo, X., Liu, A., Dong, J., Liu, B., Zhang, J. and Chen, G., Experiment on the separation of air-mixed coal bed methane in THF solution by hydrate formation. *Energy Fuels*, 2012, **26**, 4507–4513.
28. Zhao, J., Zhao, Y. and Liang, W., Hydrate based gas separation for methane recovery from coal mine gas using tetrahydrofuran. *Energy Technol*., 2016, **4**, 864–869.
29. Cai, J., Xu, C., Xia, Z., Chen, Z. and Li, X., Hydrate based methane separation from coal mine methane gas mixture by bubbling using the scale-up equipment. *Appl. Energy*; http://dx.doi.org/10.1016/j.apenergy.2017.05.010.
30. Seitz, J. C., Pasteris, J. D. and Chou, I., Raman spectroscopic characterization of gas mixtures: I. Quantitative composition and pressure determination of CH_4, N_2 and their mixtures. *Am. J. Sci*., 1993, **293**, 297–321.
31. Chari, V. D., Prasad, P. S. R. and Murthy, S. R., Structural stability of methane hydrates in porous medium: Raman spectroscopic study. *Spectrochim. Acta A*, 2014, **120**, 636–641.

ACKNOWLEDGEMENTS. We thank the Director, National Geophysical Research Institute (NGRI), Hyderabad for encouragement and permission to publish this paper. Partial financial support from DST and DGH-NGHP, New Delhi is acknowledged. This is a contribution to the GEOSCAPE Project of NGRI under the 12th Five-Year Scientific Programme of CSIR, New Delhi.

Received 23 May 2017; revised accepted 14 September 2017

doi: 10.18520/cs/v114/i03/661-666

Identification of bovine viral diarrhoea virus type 2 in cattle bull semen from southern India and its genetic characterization

Niranjan Mishra[1,*], Semmannan Kalaiyarasu[1], K. C. Mallinath[2], Katherukamem Rajukumar[1], Rohit K. Khetan[1], Siddharth Gautam[1], M. D. Venkatesha[2] and S. M. Byregowda[2]

[1]ICAR-National Institute of High Security Animal Diseases, Anand Nagar, Bhopal 462 022, India
[2]Southern Regional Disease Diagnostic Laboratory, Institute of Animal Health and Biologicals, Hebbal, Bengaluru 560 024, India

Although bovine viral diarrhoea virus (BVDV) is prevalent in Indian cattle causing economic losses in cattle farming, its detection in bull semen has not yet been reported. Following passage of raw bull semen (n = 4) on MDBK cells, testing for BVDV was done by antigen ELISA and real-time RT-PCR. BVDV type-2 (BVDV-2) was identified in three samples from southern India by real-time RT-PCR. Genetic typing of the 5′-UTR sequences classified all the three BVDV strains as BVDV-2a subtype. These were found genetically closely related to the strains from USA, but divergent from the BVDV-2a strains from northern India. Phylogenetic analysis of N^{pro} sequences confirmed the findings. The results provide evidence of circulation of BVDV-2a strains in southern India. The detection of BVDV in bull semen from India highlights the importance of mandatory testing of breeding bulls and bull semen for BVDV to minimize the risk of BVDV transmission.

Keywords: Bovine viral diarrhoea, bull semen, genetic characterization, mandatory testing.

BOVINE viral diarrhoea (BVD) is prevalent worldwide and causes substantial economic losses in cattle farming. Bovine viral diarrhoea virus (BVDV), belonging to the genus *Pestivirus* in the family Flaviviridae is the causative agent of BVD. The *Pestivirus* genus consists of four recognized species, bovine viral diarrhoea virus type-1 (BVDV-1), BNDV type-2 (BVDV-2), border disease virus (BDV) and classical swine fever virus (CSFV)[1]. The BVDV genome consists of a single stranded RNA of positive polarity and is about 12.3 kb in length. A single-open reading frame, flanked by 5′- and 3′-untranslated regions (UTRs) is translated first into a polyprotein and is then cleaved into four structural proteins (C, E^{rns}, E1 and E2) and seven to eight non-structural proteins (N^{pro}, p7, NS2-3, NS4A, NS4B, NS5A and NS5B)[2]. Sequence analysis of the 5′-UTR has been commonly used for *Pestivirus* diagnosis and classification[3–5]. Besides, N^{pro} and E2 regions of the genome are useful for detail phylogenetic analysis[3,4].

Direct or sexual contact with persistently infected (PI) cattle is the major mode of BVDV transmission. However, transmission may also occur through acutely infected animals, artificial insemination (AI), contaminated veterinary equipment and biologicals[6]. BVDV infection in bulls may result in acute infection, persistent infection and prolonged testicular infection or persistent testicular infection (PTI)[7,8]. Semen from transiently infected bulls can transmit BVDV infection and virus can be detected up to 28 days in such bulls[7]. In contrast, concentration of BVDV in both raw and extended semen of PI bulls remains high and semen from PI bulls consistently infects susceptible animals[9]. PTI develops following acute BVDV infection where bulls become nonviraemic and seropositive, but BVDV is detected in semen or testicular tissue and extended cryopreserved semen from such bulls can infect seronegative cows through AI[10,11]. Hence, semen should be tested negative for BVDV prior to its distribution for AI.

BVD is prevalent in India, and BVDV-1, BVDV-2 and BVDV-3 have been detected in cattle[5,12,13]. However,

*For correspondence. (e-mail: mishranir@rediffmail.com)

there is lack of information on BVDV circulating in cattle in southern India and virus has not yet been detected in bull semen in the country. In the present communication, we report identification of BVDV-2 originating from bull semen in southern India and determine its genetic characteristics.

A total of four infected Madin Darby bovine kidney (MDBK) cell culture supernatant samples following passage ($n = 3$) of raw bull semen along with uninfected MDBK cells were received at ICAR-National Institute of High Security Animal Diseases (NIHSAD), Bhopal from the Southern Regional Disease Diagnostic Laboratory (SRDDL), Bengaluru, for *Pestivirus* testing. The semen samples were originally collected from breeding bulls located in two farms, three from Nucleus Jerses & Stud Farm (NJF), Ooty, Tamil Nadu and one from Frozen Semen Bull Station (FSBS), Nandyal, Andhra Pradesh during April 2015 and were intended for bovine herpes virus 1 (BHV-1) testing. The bulls were cross-bred jersey and 3–4 years of age. The raw semen samples were collected in viral transport medium (VTM) and processed for isolation of BHV-1 using MDBK cells and Eagle's minimal essential medium (Sigma Aldrich, USA) as previously described[14].

All the four bull semen passaged cell culture supernatant samples and the uninfected cells were tested by *Pestivirus* antigen capture ELISA using IDEXX BVDV Ag/Serum Plus test kit (IDEXX, USA). Viral RNA extraction was carried out from 150 µl of cell culture supernatants using RNeasy mini kit (Qiagen, Germany). A *Pestivirus* generic TaqMan real-time RT-PCR assay[15] targeted at the 5′-UTR of the genome was used for detection of *Pestivirus* RNA using Light Cycler 480. The test was conducted in 25 µl reaction volume using the primers BVD190-F (ref. 15), V326 (ref. 16), probe TQ-Pesti (ref. 17), 2 µl of RNA and Superscript III Platinum one-step real-time RT-PCR reagent (Invitrogen, USA). Samples found positive for *Pestivirus* RNA were subsequently tested by TaqMan real time RT-PCR using primers and probes specific to BVDV-1, BVDV-2 and BVDV-3, using Superscript III Platinum one-step real-time RT-PCR reagent as reported earlier[18,19].

One-step RT-PCR was conducted to amplify the 5′-UTR (288 bp) using primers 324 and 326 (ref. 16) and Superscript III one-step RT-PCR System (Invitrogen, USA). To amplify the complete N^{pro} gene, cDNA synthesis followed by PCR using primers 390F (ref. 20) and 1400R (ref. 21) was done to obtain a 1080 bp product (position in BVDV strain SD1: nt 368–1448). Following purification from agarose gel, cloning of the amplified N^{pro} product was performed using the pGEMT Easy T/A cloning kit (Promega, USA), and the plasmids were sequenced using V.3.1 cycle sequencing kit (Applied Biosystems, USA) and ABI 3130 Genetic Analyzer (Applied Biosystems, USA). The 5′ UTR PCR products were subjected to sequencing directly using the primers 324 and 326.

Assembly of the corresponding overlapping sequences was performed using SeqMan II program of DNASTAR software (DNASTAR Inc., Madison, USA). Reference BVDV sequences were taken from the NCBI database and earlier works. Sequence alignment for 240 bases in the 5′-UTR and 474 bases in N^{pro} gene (416–889 nt, BVDV-2 strain 890) was carried out using Clustal W program. The percentage nucleotide identity was generated by MegAlign program of DNASTAR. Evolutionary distances, phylogenetic and bootstrap analyses were performed by neighbour-joining using the MEGA version 6.0 software[22]. The nucleotide sequences have been submitted to GenBank under the following accession numbers, 5′-UTR: Ind 3012339 – MF157328; Ind 3012340 – MF157329; Ind 3012341 – MF157330; N^{pro}: Ind 3012339 – MF157331.

BVDV antigen ELISA results showed that three of the four semen passaged cell supernatants were positive. The results of *Pestivirus* generic TaqMan assay showed that the same three (Ind 3012339, Ind 3012340 and Ind 3012341) samples were positive for pestiviruses with Cp values of 23.0–27.1. When these samples were subjected to BVDV-1, BVDV-2 or BVDV-3 specific TaqMan assay, all were found to be of BVDV-2 (Cp value range 27.41–30.37). All the positive samples originated from the bulls in Tamil Nadu. The uninfected cells tested negative for *Pestivirus* by both antigen ELISA and real-time RT-PCR.

Comparison of the 5′-UTR nucleotide sequences showed 99.6–100% identity among them. Phylogenetic analysis using the 5′-UTR sequences demonstrated that all the three strains, i.e. Ind 3012339, Ind 3012340 and Ind 3012341 belonged to BVDV-2a subtype (Figure 1) having 93.3–94.1% nucleotide identity with the BVDV-2a reference strain 890 (Table 1). They were found to be more closely related with isolates 53039, 53100 (99.6–99.2%) and USMARC-60764 (98.3%) from USA than the previously reported Indian BVDV-2a cattle isolate Ind 141353 (95–95.4%) and Indian BVDV-2a goat isolate Ind 5197 (94.6–95%). These strains clustered separately from the previously reported BVDV-2 strains from northern India. Phylogenetic analysis of N^{pro} sequences from a representative strain (Ind 3012339) confirmed the same classification as determined by 5′-UTR (Figure 1). It shared a sequence homology of 86.7–97.9%, highest with strain USMARC-60764 and lowest with strain 890 (Table 2). However, Ind 3012339 was placed in a clade separate from the earlier reported Indian cattle isolate Ind 141353 from northern India with 89.4% sequence homology.

Artificial insemination using semen contaminated with BVDV still remains one of the major sources of BVDV transmission and can have severe reproductive consequences, including high mortality reported in yearling bulls and heifers[23]. However, there is lack of data on the prevalence of BVDV in breeding bulls used for natural service or AI in India. In accordance with minimum

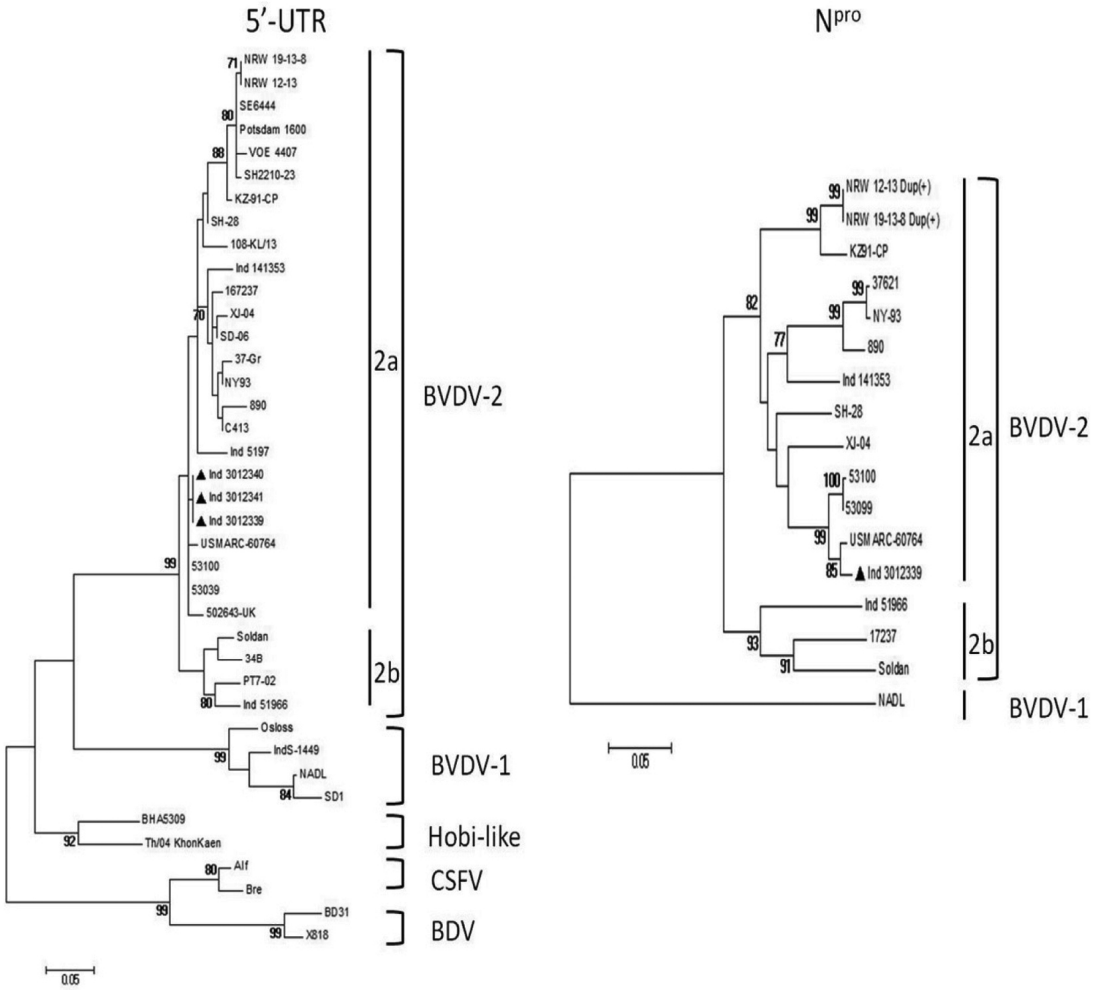

Figure 1. Genetic typing of Indian BVDV-2 strains originating from cattle bull semen in the 5′-UTR and N^{pro} regions. Numbers in nodes indicate the percentage of 1000 bootstrap replicates that support each group. BVDV strains found in this study are labelled as filled triangles.

standards of frozen bovine semen production, the bulls should be free from BVDV by virus isolation, RT-PCR or antigen capture ELISA prior to entry, during quarantine and in the semen stations and all the bulls should be tested for both acute and persistent infection[11].

A *Pestivirus* generic TaqMan assay targeted at the highly conserved 5′-UTR was used in this study, as the assay has been found useful for detecting BVDV-1, BVDV-2 and BVDV-3, including the highly divergent BVDV-3 strains in Indian cattle[5,15]. Previous studies have shown that BVDV-1 is predominantly prevalent, while the occurrence of BVDV-2 and BVDV-3 is sporadic in Indian cattle[5,12,13]. However, detection of BVDV-2 in bull semen in this study has important implications for BVDV transmission. Detection of BVDV in raw or cryopreserved extended bovine semen and associated reproductive problems following its use have been reported earlier[7,23,24].

BVDV-2 has been classified into two subtypes based on the 5′-UTR, N^{pro} and E2 gene analysis, while nomenclature of subtype BVDV-2c remains problematic at present[3,4,25–27]. So far, BVDV-2a has been detected in goats and cattle from northern India, while BVDV-2b has been reported in sheep from western India[13,25,28]. Here we report the existence of BVDV-2a in cattle in southern India, indicating the need to study BVDV prevalence and genetic diversity in this region. A very high genetic similarity was evident between the BVDV-2 strains reported here and those from USA[27], indicating a shared common ancestor. Although the epidemiological link is difficult to prove, the most feasible reason for this finding is the probable introduction from USA through import of infected live animals, biologicals or semen. In fact, India has a historical cattle trade contact with USA and frozen bovine semen is increasingly being imported in recent years for upgradation of indigenous cattle germplasm.

In conclusion, this study revealed circulation of BVDV-2a strains in southern India, which are genetically divergent from those circulating in northern India. Additionally, the study reported the detection of BVDV in bull

Table 1. Percentage of nucleotide sequence identity between BVDV-2 strains reported in this study and other BVDV-2 strains and reference pestiviruses in the 5′-UTR

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
***	94.6	94.6	92.9	77.8	70.3	92.5	89.1	94.6	**94.1**	**94.1**	**93.3**	72.6	74.1	90.4	72.8	94.6	87	93.3	65.7	95	1. 890
	***	100	97.5	77	72.5	95.4	90.9	95.8	**99.6**	**99.6**	**99.2**	72.6	77.4	95.1	76	98.4	92.2	98.8	66.3	96.2	2. 53039
		***	97.5	77	72.5	95.4	90.9	95.8	**99.6**	**99.6**	**99.2**	72.6	77.4	95.1	76	98.4	92.2	98.8	66.3	96.2	3. 53100
			***	74.6	73.3	93.8	92.1	93.8	**97.1**	**97.1**	**96.7**	73.4	75.8	93.3	74.6	95.8	92.1	96.2	69.6	93.8	4. 502643-uk
				***	72.9	75.8	73.1	77.1	**76.2**	**76.2**	**76.2**	71.7	70.4	76.5	71.1	76.1	76.5	75.8	70	77.1	5. BHA5309 [BVDV-3]
					***	72.5	71.2	72	**72.5**	**72.5**	**72**	71.6	73.3	72.9	74.2	71.6	74.6	72	81.8	72	6. CSFV_Alf
						***	88.8	93.3	**95**	**95**	**94.6**	71.7	73.8	92.1	73.8	95.4	89.6	94.2	67.5	93.3	7. Ind 5197
							***	89.6	**91.2**	**91.2**	**90.8**	72.2	74.8	89.3	74	90.9	93	90.4	69	90	8. Ind 51966
								***	**95.4**	**95.4**	**95**	73.8	77.1	92.9	75.8	95.8	90.4	94.6	67.1	96.2	9. Ind 141353
									***	**100**	**99.6**	**72.2**	**76.7**	**94.6**	**75**	**97.9**	**92.9**	**98.3**	**67.1**	**95.8**	**10. Ind 3012339**
										***	**99.6**	**72.2**	**76.7**	**94.6**	**75**	**97.9**	**92.9**	**98.3**	**67.1**	**95.8**	**11. Ind 3012340**
											***	**71.3**	**76.2**	**93.7**	**75.3**	**97.1**	**92.5**	**98.3**	**67.8**	**95.8**	**12. Ind 3012341**
												***	89.9	72.2	90.3	73	73.4	71.7	70.9	73.4	13. IndS-1449
													***	78.6	90.9	77	77	76.2	71.6	74.6	14. NADL
														***	74.4	95.9	88.5	93.8	67.7	92.9	15. NRW 12-13
															***	76	75.2	75.4	69.8	75.4	16. Osloss
																***	91.4	97.1	66.3	96.2	17. SH-28
																	***	92.9	69.3	90.8	18. Soldan
																		***	67.1	95.8	19. USMARC-60764
																			***	67.9	20. 818
																				***	21. XJ-04

Table 2. Percentage of nucleotide sequence identity between BVDV-2 strains reported in this study and other BVDV-2 strains and reference pestiviruses in N^{pro}

1	2	3	4	5	**6**	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
***	80.3	83.9	83.5	86.7	**82.2**	65.5	82.2	81.7	65.8	83.3	83.3	80.8	69.1	67	82	87.9	85.2	80.3	83.7	1. 17237
	***	89.4	89	82.2	**86.7**	70.6	90.7	86	66.4	87.7	87.7	99.4	72.1	68.1	88.4	79.1	87.3	96.4	87.5	2. 37621
		***	99.6	84.1	**96.2**	68.7	90.5	89.1	66.8	90.1	90.1	89	71	68.3	91.1	82.9	92.4	88.4	96.4	3. 53099
			***	83.7	**95.8**	68.7	90.1	88.9	67	89.6	89.6	88.6	71	68.5	90.7	82.5	92	87.9	96	4. 53100
				***	**83.3**	67	82.2	81.7	65.8	84.4	84.4	82.2	67.9	67.9	83.5	84.8	85	83.1	83.5	5. Ind 51966
					***	**68.3**	**89.4**	**88**	**65.5**	**88.2**	**88.2**	**86.9**	**68.7**	**66.2**	**89.9**	**82.2**	**91.3**	**86.7**	**97.9**	**6. Ind 3012339**
						***	68.7	67.4	75.3	68.7	68.7	70	84.8	77.6	68.9	66	68.3	69.1	68.3	7. Ind S1449
							***	87.7	68.9	88.8	88.8	90.7	71.9	68.1	90.1	80.1	89.9	89.6	89.4	8. Ind141353
								***	67.7	96.3	96.3	86	68	68	89.1	80.3	89.1	86.6	88	9. KZ91-CP
									***	68.7	68.7	66.8	79.1	91.5	68.3	67.9	66.6	67.9	65.5	10. NADL
										***	100	87.7	72.5	70.2	89.4	81	89.9	88.2	88.6	11. NRW 12-13_Dup(+)
											***	87.7	72.5	70.2	89.4	81	89.9	88.2	88.6	12. NRW 19-13-8_Dup(+)
												***	71.9	67.7	89	79.1	87.3	96.2	87.5	13. NY-93
													***	80.1	72.5	68.7	71.7	72.3	70	14. Osloss
														***	70	68.5	68.5	69.1	66.6	15. SD1
															***	80.3	91.5	89	90.3	16. SH-28
																***	83.1	79.5	82.9	17. Soldan
																	***	87.1	92.2	18. strain XJ-04
																		***	87.3	19. 890
																			***	20. USMARC-60764

semen from India, which highlights the importance of mandatory testing of breeding bulls and bull semen for BVDV to minimize the risk of transmission. Identification of BVDV-2 in bull semen emphasizes the need for continued BVDV surveillance and serious efforts for implementation of minimum standards for the production of bovine semen in India.

1. King, A. M. Q., Adams, M. J., Carstens, E. B. and Lefkowitz, E. J., Virus taxonomy: classification and nomenclature of viruses. In *Ninth Report of the International Committee on Taxonomy of Viruses*, Academic Press, San Diego, CA, USA, 2011, pp. 1003–1020.
2. Meyers, G. and Thiel, H. J., Molecular characterization of pestiviruses. *Adv. Virus Res.*, 1996, **47**, 53–118.
3. Vilcek, S. *et al.*, Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. *Arch. Virol.*, 2001, **146**, 99–115.
4. Becher, P. *et al.*, Genetic and antigenic characterization of novel pestivirus genotypes: implication for classification. *Virology*, 2003, **311**, 96–104.
5. Mishra, N. *et al.*, Identification and molecular characterization of novel and divergent HoBi-like pestiviruses from naturally infected cattle in India. *Vet. Microbiol.*, 2014, **174**, 239–246.
6. Niskanen, R. and Lindberg, A., Transmission of bovine viral diarrhoea virus by unhygienic vaccination procedures, ambient air, and from contaminated pens. *Vet. J.*, 2003, **165**, 125–130.
7. Kirkland, P. D., Richards, S. G., Rothwell, J. T. and Stanley, D. F., Replication of bovine viral diarrhoea virus in the bovine reproductive tract and excretion of virus in semen during acute and chronic infections. *Vet. Rec.*, 1991, **128**, 587–590.
8. Grooms, D. L., Reproductive consequences of infection with bovine viral diarrhoea virus. *Vet. Clin. North Am. Food Anim. Pract.*, 2004, **20**, 5–19.
9. Kirkland, P. D., MacIntosh, S. G. and Mole, A., The outcome of widespread use of semen from a bull persistently infected with pestivirus. *Vet. Rec.*, 1994, **135**, 527–529.
10. Voges, H., Horner, G. W., Rowe, S. and Wleenberg, G. J., Persistent bovine pestivirus infection localized in the testes of an immuno-competent, non-viremic bull. *Vet. Microbiol.*, 1998, **61**, 165–175.
11. Givens, M. D. *et al.*, Noncytopathic bovine viral diarrhoea virus can persist in testicular tissue after vaccination of peri-pubertal bulls but prevents subsequent infection. *Vaccine*, 2007, **25**, 867–876.
12. Mishra, N. *et al.*, Genetic typing of bovine viral diarrhoea virus isolates from India. *Vet. Microbiol.*, 2004, **104**, 207–212.
13. Behera, S. P. *et al.*, Genetic and antigenic characterization of bovine viral diarrhoea virus type 2 isolated from cattle in India. *Comp. Immunol. Microbiol. Infect. Dis.*, 2011, **34**, 189–196.
14. OIE, Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Office International des Epizooties, Paris, 2016, chapter 2.4.7, pp. 1–22.
15. Hoffmann, B., Depner, K., Schirrmeier, H. and Beer, M., A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses. *J. Virol. Methods*, 2006, **136**, 200–209.
16. Vilcek, S., Herring, A. J., Herring, J. A., Nettleton, P. F., Lowings, J. P. and Paton, D. J., Pestiviruses isolated from pigs, cattle and sheep can be allocated into at least three genogroups using polymerase chain reaction and restriction endonuclease analysis. *Arch. Virol.*, 1994, **136**, 309–323.
17. Gaede, W., Reiting, R., Schirrmeier, H., Depner, K. R. and Beer, M., Detection and species-specific differentiation of pestiviruses using real-time RT-PCR. *Berl. Muench. TierarztlWochenschr*, 2005, **118**, 113–120.
18. Baxi, M., McRae, D., Baxi, S., Greiser-Wilke, I., Vilcek, S., Amoako, K. and Deregt, D., A one-step multiplex real time RT-PCR for detection and typing of bovine viral diarrhoea viruses. *Vet. Microbiol.*, 2006, **116**, 37–44.
19. Liu, L., Xia, H., Belak, S. and Baule, C., A TaqMan real-time RT-PCR assay for selective detection of atypical bovine pestiviruses in clinical samples and biological products. *J. Virol. Methods*, 2008, **154**, 82–85.
20. Nagai, M. *et al.*, Phylogenetic analysis of bovine viral diarrhoea viruses using five different genetic regions. *Virus Res.*, 2004, **99**, 103–113.
21. Becher, P., Orlich, M., Shannon, A. D., Horner, G., Konig, M. and Thiel, H. J., Phylogenetic analysis of pestiviruses from domestic and wild ruminants. *J. Gen. Virol.*, 1997, **78**, 1357–1366.
22. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., MEGA6: Molecular evolutionary genetics analysis version 6.0, *Mol. Biol. Evol.*, 2013, **30**, 2725–2729.
23. Gonzalez Altamiranda, E. A. *et al.*, Clinical and reproductive consequences of using BVDV-contaminated semen in artificial insemination in a beef herd in Argentina. *Anim. Rep. Sci.*, 2012, **133**, 146–152.
24. Givens, M. D., Heath, A. M., Brock, K. V., Brodersen, B. W., Carson, R. L. and Stringfellow, D. A., Detection of bovine viral diarrhoea virus in semen obtained after inoculation of seronegative postpubertal bulls. *Am. J. Vet. Res.*, 2003, **64**, 428–434.
25. Mishra, N., Rajukumar, K., Vilcek, S., Tiwari, A., Satav, J. S. and Dubey S. C., Molecular characterization of bovine viral diarrhoea virus type 2 isolate originating from a native Indian sheep (*Ovis aries*). *Vet. Microbiol.*, 2008, **130**, 88–98.
26. Jenckel, M., Höper, D., Schirrmeier, H., Reimann, I., Goller, K. V., Hoffmann, B. and Beer, M., Mixed triple: allied viruses in unique recent isolates of highly virulent type 2 bovine viral diarrhoea virus detected by deep sequencing. *J. Virol.*, 2014, **88**, 6983–6992.
27. Workman, A. M. *et al.*, Resolving bovine viral diarrhoea virus subtypes from persistently infected U.S. beef calves with complete genome. *J. Vet. Diagn. Invest.*, 2016, **28**, 519–528.
28. Mishra, N., Dubey, R., Rajukumar, K., Tosh, C., Tiwari, A. and Pitale, S. S., Genetic and antigenic characterization of Indian bovine viral diarrhoea virus type 2 isolated from Indian goats (*Capra hircus*). *Vet. Microbiol.*, 2007, **124**, 340–347.

ACKNOWLEDGEMENTS. The funding for this study was provided by ICAR-NIHSAD, Bhopal. We thank the Director, ICAR-NIHSAD, for providing infrastructure facilities.

Received 14 December 2016; revised accepted 19 September 2017

doi: 10.18520/cs/v114/i03/666-670