Circulation of classical swine fever virus (CSFV) strains of bovine origin in China and India

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Summary

The classical swine fever virus (CSFV) is a species member of the family *Flaviviridae*. CSFV is widely distributed in the world causing a severe impact on pig industry. This pathogen is considered restricted to domestic and wild suids. However, some reports from 2014 to 2018 showed the presence of the CFSV antigen in the bovine species. The virus was found in commercialized batches of fetal bovine serum (FBS) of Chinese origin and in bovine herds in in the provinces of Henan and Jiangsu, China, and in Tamil Nadu and Meghalaya, southern and north-eastern states of India, respectively. Detection was done using antigen capture ELISA and RT-PCR tests. In certain cases, animals with natural infection showed clinical signs and reproduction was also affected.

Genetic characterization was performed considering the 5'-UTR sequences of the bovine strains. In addition, the entire CSFV E2 genomic region could be amplified from two positive animals. The bovine strains were genetically related to the Chinese CSFV live attenuated hog cholera lapinized vaccine (HCLV) strain used in pigs, sharing sequence characteristics. The vaccine strain HCLV was widely used in China to protect bovines and yaks from bovine viral diarrhea, and, as a possible consequence, inducing an adaptation in cattle and a further natural diffusion. Furthermore, a contaminant strain from China was genetically distant from all other previously described genotypes of the CSFV. This suggests also the occurrence of micro evolutive step in the species related to geographical segregation.

These observations deserve attention and further investigations, especially relevant in countries where CSFV control and eradication strategies are applied.

Introduction

Pestiviruses, of the family *Flaviviridae*, are genetically related species which are recognized important pathogens in the veterinary medicine. Among these species, apart recent eradication strategies applied by some European countries against bovine viral diarrhea virus (BVDV), the main attention was focused for several years on the classical swine fever virus (CSFV) and this resulted in the eradication of the disease in different countries as in Europe and North America. Nevertheless, nowadays control and eradication against the CSFV are still a priority in various other regions. The disease is regulated by the code of the World Organization for Animal Health (Office International des Epizooties - OIE), based on the mandate by the World Trade Organization, with specific restrictive international rules for the trade of live animals, products of animal origin and germinal products.

It is generally accepted, as a consensus, that pestiviruses are characterized by the capacity to cross species barrier. Often field surveys in various countries report of the circulation of pestiviruses in

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new hosts species. This is true in particular for BVDV, described in new camelids or in exotic animals. In addition, studies demonstrate the increasing heterogeneity in the genus, indicating the high potential of adaptation to the environment and new hosts.

Pestiviruses have a wide host range and this may result in the creation of new reservoirs in domestic species or wildlife, a possible problem for the sustainability of eradication campaigns. Apparently, such issue was not relevant for CSFV since the virus was generally considered to be restricted to domestic and wild suids. Experimental infections in cattle, sheep, goats and deer with CSFV did not produce clinical symptoms and no natural infection of these species was reported (Loan and Storm, 1968; Biró *et al*., 1966; Shimizu and Kumagai, 1989; CFSPH, 2015). This led to be believe that CSFV was an exception among pestiviruses, characterized by an epidemiological static nature and unable to cross species barrier in natural conditions. However, in recent years, isolations of CSFV strains in cattle (B*os taurus*) were reported from India and China. The virus was found as contaminant of bovine serum or associated to natural infection with clinical course and reproductive disorders (Zhang *et al*., 2014; Giangaspero *et al*., 2017; Chakraborty *et al*., 2018; Giangaspero and Zhang, 2020). Here, we briefly describe the observations reported in these countries.

Observations in India

During a field screening for the identification of persistently infected animals by BVDV in bovine and buffalo farms from 4 districts in the state of Tamil Nadu, India, 12 animals were seropositive to BVDV by enzyme linked immunosorbent assay (ELISA) and positive to BVDV antigen by reverse transcription polymerase chain reaction (RT-PCR). The BVDV strains were isolated from individuals of six months to two years of age, in 4 farms, out of 19 tested (Giangaspero *et al*., 2017). Interference due to vaccination was excluded since no prophylactic protocols implying immunization against BVDV were applied in the studied area. Furthermore, immune reactivity and antigenic positivity was not due to virus circulation in pigs since none of the cattle herds screened showed having contact with pigs. All the 12 cattle CSFV strains originated from animals suffering from reproductive disturbances such as abortions, congenital malformations, repeat breeding and mastitis, suggesting a direct relation between positivity to CSFV and clinical pathologies. Differential diagnosis could exclude bovine herpesvirus 1 (IBR) and *Brucella* sp. (serological positivity absent or very low, manifestly not correlated to observed reproduction disorders). Animals were also negative for BVDV (Giangaspero *et al*., 2017).

In addition, a further screening conducted in the state of Meghalaya, northeast of India. 134 bovine serum samples were positive by RT-PCR for 5′ untranslated region (UTR) region of CSFV and 10 samples reacted positive for CSFV antigen using a commercial antigen capture ELISA (Chakraborty *et al*., 2018). In two positive samples the full length E2 region of CSFV was amplified.

Observations in China

In China, positivity against CSFV antigen were observed in screenings in cattle farms to investigate etiological causes of health problems and in the framework of regular checks of commercialized batches of bovine fetal serum (BFS) to detect adventitious BVDV contaminants. Despite no live viruses have been isolated, four CSFV strain sequences were obtained during these screenings.

The sequence of strain HEN03 [KC176778] was detected in a bovine originating from the province of Henan (Giangaspero and Zhang, 2020). The CSFV strain HEN03 was isolated in 2012, and this was the first description of the virus in cattle in China. The positive animal belonged to a herd suffering from reproductive disturbances. Many animals in the herd showed abortion, stillbirth and reduced milk yield. Also, mortality rate was high. However, pathologies could not be necessarily referred to CSFV since concomitant infections with BVDV-1 and BVDV-2 were also present.

The 3 CSFV RNA sequences detected from BFS batches were designated as strains S171 [KF006974], S173 [KF006975] and S112 [MK118725] (Zhang *et al*., 2014). They have been obtained during a study aiming to determine the genetic diversity of contaminant BVDV in commercialized BFS of Chinese origin, by RT-PCR and sequencing (Zhang *et al*., 2014). The testing was performed on 22 batches of BFS obtained from 10 suppliers from different geographic locations in the country. Positivity was very high (20 out 22 tested). In addition to the 3 sample sequences (strains S171, S173, and S112), originated from Henan and Jiangsu provinces, clustered into CSFV, the phylogenetic reconstructions of partial 5′UTR sequences indicated that the other 17 samples belonged to the BVDV type 1 and BVDV type 2 species.

Genetic characterization

Based on primary structure analysis, using the neighbor-joining method (Saitou and Nei, 1987), a phylogenetic tree was constructed from the alignment of the 5´-UTR sequences of representative

strains of CSFV genotypes (Figure 1) to determine the taxonomic status of the strains isolated in cattle at the level of CSFV species.

The genomic characterization of the strains of bovine origin was also performed according to secondary structure evaluation of the internal ribosome entry site (IRES), in addition to the consideration of primary structure analysis. The bovine strains were clustered into genotypes within the CSFV species using the palindromic nucleotide substitution (PNS) method (Harasawa and Giangaspero, 1998; Giangaspero and Harasawa, 2007; Giangaspero and Apicella, 2014), through the evaluation of the 5'-UTR of RNA sequence. The PNS method was previously successfully applied to characterize also atypical pestiviruses, such as the strains Giraffe, Pronghorn or Bungowannah (Harasawa *et al*., 2000; Giangaspero and Harasawa, 2011). The qualitative and quantitative procedure of the PNS method considered the relevant genomic sequences of the 3 variable loci, V1, V2 and V3, in the IRES in the secondary structure identifiable at the level of the full length (216-237 nt) 5'-UTR of the viral RNA, the for the taxonomic allocation into CSFV species clusters. This allowed the identification of genotypes, based on the different combinations of nucleotide base-pairs (bp) in the low-variable positions of the 3 loci. Genogroups were subsequently ranked according to their increased divergence in the species, and defined using an alphabetic nomenclature.

The 13 sequences originating from bovines in India and China and the 3 sequences identified in commercial BFS batches from China (Zhang *et al*., 2014; Giangaspero *et al*., 2017; Giangaspero and Zhang, 2020) were compared with other 110 5'-UTR CSFV strains (field swine isolates, vaccine strains, adventitious contaminants of swine cell lines and an ovine isolate from Spain) representative of the different genetic groups in the CSFV species, including strains circulating in India, China and other Asian countries. They were also subsequently compared to about 1,400 RNA sequences from *Pestivirus* species other than CSFV.

The genetic evaluation of the strains isolated in China and India by 5'-UTR secondary structure analysis indicated their appurtenance to CSFV species. The 3 Chinese strains belonged to the Alfort type CSFV genotype (CSFV-a2) and all the Indian strains were of the Brescia type CSFV genotype (CSFV-a1). The Indian sequences were highly homogeneous, with full identity at the level of the V2 and V3 loci. The bovine strains isolated in Tamil Nadu (southern India) were genetically related to CSFV strains circulating in pigs in the northern India.

Only one strain from China, the strain S171, represented a new CSFV genotype (PNS D). The 5'-UTR sequence was partially related to strains of the genotype CSFV-a2. However, the S171strain showed a marked difference of the V3 locus from all other CSFV clusters. Another relevant characteristic of this strain was the significantly low relation with the Border disease virus (BDV) species, which is a known peculiarity of the CSFV strains. The divergence values obtained comparing S171 and strains of the BDV genotypes where well higher than those resulting from the comparison of the other CSFV strains to BDV.

The characterization by PNS method of 2 sequences of the Indian CSFV strains CS/ML/911/IDP/13 [KY860532] and CS/ML/AF/Umiam/14 [KY860531], reported in cattle by Ahuja *et al*. (2015) and Chakraborty *et al*. (2018), for which was available only the full length E2 region, was made indirectly, taking into account their relatedness with other previously deposited pig sequences. Their E2 regions were similar to pig strains as IND/AS/GHY/G4 [KM362426] (Ahuja *et al*., 2015), isolated in Assam, a neighboring northern state of India. Using the web-based sequence analysis tool BLAST (http://www.ncbi.nlm.nih.gov), an overall nucleotide homology of 92% to 99% was obtained for the E2 sequences among strains CS/ML/911/IDP/13, CS/ML/AF/Umiam/14 and IND/AS/GHY/G4 (CSFV-a5) with the CSFV reference strain Paderborn [GQ902941] (CSFV-a2). Considering the clustering in the species of the strains Paderborn and IND/AS/GHY/G4 by PNS on their 5'-UTR, the strains CS/ML/911/IDP/13 and CS/ML/AF/Umiam/14 of bovine origin were allocated into a same genotype, characteristic to India.

The genomic groups visualized by a phylogenetic tree, based on primary structure analysis (Figure 1) were similar to the genotypes defined by PNS (secondary structure analysis). This subdivision corresponded to the definition made by other authors, dividing CSFV species into three major lineages (Brescia, Alfort and Kanagawa/Okinawa types) and their sub lineages (Lin *et al*., 2007). It was also evident the adaptation of CSFV in new bovine host, showing new genomic traits peculiar of bovine origin, with stable nucleotide sequences characteristics, expression of evolutive steps in the history of the virus. The CSFV species showed a low heterogeneity with a relatively low number of genetic clusters. This was in contrast with other *Pestivirus* species as the BVDV type 1 with at least 25 genotypes (Giangaspero and Zhang, 2023). Among the CSFV genotypes, CSFV-c and CSFV-d, both reported only in Asian countries, presented high divergence in the species. Due to this high divergence in the species, the strain Kanagawa/74 (CSFV-c) isolated in Japan in 1974 (Dreier *et al*., 2007; Beer *et al*., 2015) was previously proposed as outgroup strain, along with Congenital Tremor (United Kingdom, 1964), for phylogenetic studies (Paton *et al*., 2000). Similarly, the high divergence in

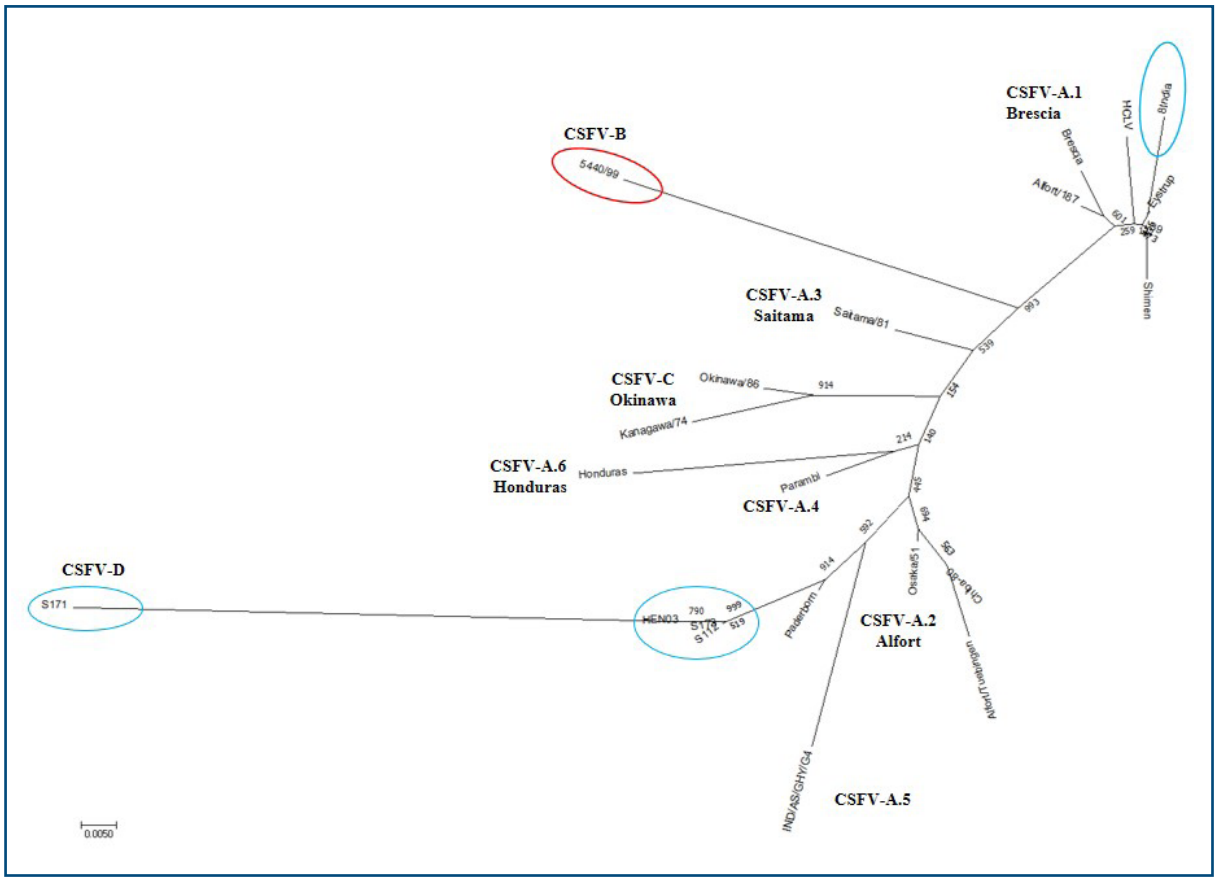

Figure 1. *Phylogenetic tree based on the 5'-UTR of CSFV strains in the Pestivirus genus (Giangaspero and Zhang, 2020). Genotypes including strains of bovine origin are indicated in blue circles. Genotypes including strains of ovine origin are indicated in red circles. Distances were computed using Clustal X (Chenna* et al.*, 2003), version 1.8, using the neighbor-joining method (Saitou and Nei, 1987). Scale bar indicates 10 nucleotide substitutions per 100 nucleotides.*

the CSFV species showed by the bovine strain S171 (CSFV-d) (Zhang *et al*., 2014), observed only in China, should justify its consideration as another outgroup of CSFV, with more enhanced divergence.

Exception made for strain S171, the other bovine isolates from China were related to common CSFV strains belonging to the genotype CSFV-a2 (type Alfort), thus a simultaneous circulation of typical and atypical CSV types, the latter possibly related to an adaptation to a novel animal host species or due to geographic segregation. In China, geographic segregation was previously suggested in relation to atypical BVDV-1 strains (Xue *et al*., 2010; Wang *et al*., 2014).

No atypical sequences were observed among the cattle isolates from India. They were genetically related to typical strains belonging to CSFV genotype a variant 1 (type Brescia) and were similar to the virus population circulating in pigs reported in India. In particular, pig strains Aizawl-09 [HM449066], 5NCR/CSF/MZ/AIZ/348 [JX975460] and 5NCR/CSF/MZ/AIZ/352 [JX975461] (Rajkhowa *et al*., unpublished) resulted genetically closely related to the strains reported in cattle. They were isolated in 2009 and 2011 in Mizoram, at the extreme north eastern border with Myanmar, very distant from the southern state of Tamil Nadu, from where cattle resulted positive to CSFV. However, despite the appurtenance to the same genotype, the Indian bovine strains where characterized by a distinctive guanine uracil (G*U) base pairing in position 3 in the V1 locus. In all other CSFV strains, a conserved A-U pairing is present in this position. Interestingly, the base pairing observed in the V1/3 in the sequences of the bovine Indian strains is shared only with the bovine strains of the BVDV species type 1, 2 and 3, where G-C or G*U pairings are highly conserved.

While current CSFV taxonomy relies on the consideration of the characteristics of the E2 genomic region (Postel *et al*., 2012), the laboratory tests (primary and secondary structure analyses) used to evaluate the cattle strains were based on the 5'-UTR. The choice for accurate phylogenetic analyses of the full-length E2 encoding sequences (1,119 nucleotides) is due to its relation to the glycoprotein of the envelope (main immunogen and essential for CSFV vaccine preparation) (Perez *et al*., 2012). The characterization of CSFV types and subtypes is also based on a 190 nucleotide fragment of the E2 glycoprotein (Dreier *et al*., 2007). Other

short length target fragments in other regions of the viral genome were considered less suitable to differentiate closely related sequences (Postel *et al*., 2012). However, despite this limiting factor, short length target fragments, such as the 5'-UTR or the NS5B, have been largely used to determine genotypes and molecular epidemiology (Lowings *et al*., 1996; Paton *et al*., 2000; Beer *et al*., 2015). Furthermore, short length target fragments are contemplated among CSFV reference tests indicated by the World Organization for Animal Health (Office International des Epizooties - OIE), the main authority on laboratory testing international standards. The E2 glycoprotein gene (190 nucleotides) and the 5'-UTR (150 nucleotides) both they are admitted for molecular epidemiology and genotyping of the CSFV species by the OIE Terrestrial Manual (Chapter 2.8.3; Paragraph 1.1.5.) (OIE, 2014). Also the method used to characterize the bovine CSFV strains (Zhang *et al*., 2014; Giangaspero *et al*., 2017), RT-PCR amplification followed by nucleotide sequencing, is admitted among recommended laboratory tests for CSFV agent detection, confirmation of clinical cases and differentiation from other pestiviruses, according to the OIE Terrestrial code (Chapter 15.2; Article 15.2.28, Paragraph 3) (OIE, 2017) and the OIE Terrestrial Manual (Chapter 2.8.3; Paragraph 1.1.4.) (OIE, 2014). Despite the preference to evaluate the gene encoding the E2 glycoprotein sequence for CSFV species taxonomy (Postel *et al*., 2012), the consideration of the 5'-UTR was the only possible approach to apply the PNS method and in order to compare CSFV with other *Pestivirus* species, otherwise not feasible giving that PNS analyses the IRES and most of the deposited sequences of pestiviruses are 5'-UTR (Giangaspero *et al*., 2018).

Source of infection

Prior to the detection of CSFV in bovines in India and China, only two reports described the occurrence of the virus in animal host species different from domestic and wild pigs, corroborating the idea that natural infection was restricted to suids. In Spain, a deposited ovine 5'-UTR sequence (strain 5440/99) was related to CSFV vaccine strains used in pigs (Hurtado *et al*., 2003). This suggested a relation with prophylactic campaigns in pig farms and spillovers as a consequence. In a second occasion, during investigations on pestiviruses in the United Arab Emirates the strain 12 Ovine liver 113nt was isolated from an ovine sample (Mohamed, 2004). The sequence was considered a *Pestivirus*-like, showing a certain homology with different pestiviruses by alignment. The greatest similarity was obtained with strains of the CSFV sub genotype 1.1. However, it was not possible to obtain a correct classification, due to the suboptimal quality of the sequence and the short length fragment of the 5'-UTR (113 nucleotides) (Mohamed, 2004). In Belgium, during the 90ties, CSFV-like 5'-UTR sequences have ben detected in some samples of BFS (Giangaspero and Zhang, 2020). The experimental inoculation of these samples in pigs was unable to determine infection or seroconversion, suggesting the occurrence of an unidentified virus possessing sequence similarities with CSFV, within the 5'-UTR. It cannot be excluded that, as suspected in Spain, also in Belgium wild and vaccinal strains where present in the country (last occurrence of CSFV in 1997) and spillovers occurred in cattle from naturally infected or vaccinated pigs.

Following these previous rare reports, the observations in cattle populations in Asia and the Indian subcontinent showed a clearer epidemiological link with CSFV in pigs. In both countries, bovine CSFV strains (CSFV type 1 and 2, Brescia and Alfort, respectively) were associated with the concomitant circulation of genetically related strains in pigs.

Similar strains were reported also as adventitious contaminants of biological products for veterinary use and used as vaccine strains (CSFV type 1, Brescia). In China, CSFV strains were first reported in cattle in 2012, while pig strains belonging to the same genotype were circulating since 2009 to 2012, and than reported as contaminants in 2014. CSFV pig strains were reported in China since 1999 to 2009, and circulated in India since 2007 to 2013, reported as contaminants in 2008 and 2011 or used as vaccine in different countries as China, Japan or Russia, than CSFV strains were detected in cattle in India in 2016 (Table I), suggesting the diffusion of CSFV in cattle through different ways. Both primary and secondary genomic structure analysis showed a genetic similarity between the strains of bovine origin from China and India with the CSFV lapinized vaccine strain (live attenuated hog cholera lapinized vaccine - HCLV), suggesting the origin of CSFV in cattle to be related to vaccine prophylaxis performed in pig populations.

In India, in the farms investigated in Tamil Nadu, vaccines against CSFV or BVDV were not used (Giangaspero *et al*., 2017). However, prophylaxis against CSFV is applied in the swine industry in the country. Interestingly, the genotype CSFV-a1 includes a lapinized vaccine strain from India (EU857642 - AF091507) which showed 99% homology with the Indian bovine strains by BLAST.

In China, before the introduction of BVDV inactivated virus vaccines, for long time, bovines and yaks were protected against BVDV through the frequent and diffuse application of a triple dose of the HCLV vaccine in different Chinese provinces, in particular in Tibet and Qinghai. In Tibet, the losses caused by BVDV infection were reduced by the use of HCLV. This

use was based on the experimental study conducted by Yuan Qingzhi in 1957 which demonstrated the efficacy and safety of the prophylactic use of HCLV against BVDV in lactating and pregnant cows, calves and yaks (Liu *et al*., 2003). In addition, HCLV was for long time the unique CSFV vaccine for pigs authorized in China. Probably, the long-term use of HCLV created the optimal conditions for the vaccinal strains to adapt in bovines, and consequently allowing a further natural diffusion in the new animal host species population. Concerning the link of the observations between China and India, an element of interest might be that historically Tibet is a breeding region of high quality, based on traditional semi nomadic agropastoral system. From Tibet, live animals are seasonally traded and moved to other regions and this may cause the spread of pathogens including pestiviruses also between China and India, especially by immunotolerant persistently infected animals.

Table I. *Strains (n 59) clustered into genotypes of the CSFV species, according to PNS method (nomenclature is based on divergence in the genus), genotypes by depositors are under parenthesis. Fifteen strains are from cattle (*Bos taurus*); 31 from pigs (*Sus scrofa domesticus*); 1 from sheep (*Ovis aries*); 5 are contaminants of BFS; 7 are vaccine strains (Giangaspero and Zhang, 2020).*

Genotype	Strain	Origin	Year	Country	Accession	Reference
A1	1 India	Cattle	2016	India	MG859286	Giangaspero *et al*., 2017
A1	2 India	Cattle	2016	India	MK105825	Giangaspero *et al*., 2017
A1	3 India	Cattle	2016	India	MK105826	Giangaspero *et al*., 2017
A1	4 India	Cattle	2016	India	MK105820	Giangaspero *et al*., 2017
A1	5 India	Cattle	2016	India	MK105821	Giangaspero *et al*., 2017
A1	6 India	Cattle	2016	India	MK105822	Giangaspero *et al*., 2017
A1	7 India	Cattle	2016	India	MK109913	Giangaspero *et al*., 2017
A1	8 India	Cattle	2016	India	MG813566	Giangaspero *et al*., 2017
A1	9 India	Cattle	2016	India	MK105827	Giangaspero *et al*., 2017
A1	10 India	Cattle	2016	India	MK105823	Giangaspero *et al*., 2017
A1	11 India	Cattle	2016	India	MG859287	Giangaspero *et al*., 2017
A1	13 India	Cattle	2016	India	MK105824	Giangaspero *et al*., 2017
A1	Bangalore Ind-163/07	Pig	2007	India	EU446419	Patil *et al*., unpublished
A1	Ind-173/08	Pig	2008	India	FJ183444	Patil *et al*., 2010
A1	Ind-174/08	Pig	2008	India	FJ183445	Patil *et al*., 2010
A1	Ind-175/08	Pig	2008	India	FJ183446	Patil *et al*., 2010
A1	Ind-176/08	Pig	2008	India	FJ183447	Patil *et al*., 2010
A1	Ind-239/08	Pig	2008	India	FJ183449	Patil *et al*., 2010
A1	Ind-243/08	Pig	2008	India	FJ183452	Patil *et al*., 2010
A1	Ind-272/08	Pig	2008	India	FJ183456	Patil *et al*., 2010
A1	Aizawl-09	Pig	2009	India	HM449066	Rajkhowa *et al*., unpubl
A1	CSF/MZ/KOL/73	Pig	2009	India	JX094153	Rajkhowa, unpublished
A1	CSF/MZ/SAI/76	Pig	2009	India	JX094154	Rajkhowa, unpublished
A1 (1.1)	CSFV/IVRI/VB-131	Pig	2009	India	KM262189	Kamboj *et al*., 2014
A1	5NCR/CSF/MZ/AIZ/348	Pig	2011	India	JX975460	Rajkhowa *et al*., unpubl
A1	5NCR/CSF/MZ/AIZ/352	Pig	2011	India	JX975461	Rajkhowa *et al*., unpubl
A1 (1.1)	NFP/AS-1	Pig	2011	India	KC617749	Roychoudhury *et al*, unpub
A1 (1.1)	NFP/ML-2	Pig	2011	India	KC617761	Roychoudhury *et al*, unpub
A1 (1.1)	NFP/ML-4	Pig	2011	India	KC617750	Roychoudhury *et al*, unpub
A1 (1.1)	CSFV212L-13	Pig	2013	India	KY860615	Tomar *et al*., unpublished
A1	Shimen	Pig	1999	China	AF092448	Huang *et al*., unpublished
A1	39	Pig	2001	China	AF407339	Wu *et al*., unpublished
A1	cF114	Pig	2001	China	AF333000	Mingxiao *et al*., unpubl
A1	SWH	Pig	2004	China	DQ127910	Li *et al*., 2006

Genotype	Strain	Origin	Year	Country	Accession	Reference
A1 (1.1)	JL1(06)	Pig	2006	China	EU497410	Qiu *et al*., unpublished
A1 (1.1)	CSFV-GZ-2009	Pig	2009	China	HQ380231	Shen *et al*., 2011
A1 (1.1)	Alfort 187	Pig		France	X87939	Ruggli al., 1995
A1 (1.1)	HCLV	Vaccine		India	AF091507	Wang *et al*., unpublished
A1	GPE (-)	Vaccine		Japan	AB019152	Harasawa Giangaspero 99
A1	C strain	Vaccine	1994	China	Z46258	Moormann *et al*., 1996
A1	Rovac	Vaccine	1994	USA	KJ873238	Zhou *et al*., 2014
A1 (1.2)	RUCSFPLUM	Vaccine		USA	AY578688	Risatti *et al*., 2005
A1	KC	Vaccine		Russia	AF099102	Grebennikova *et al*., 1999
A1	LK-VNIVViM	Vaccine		Russia	KM522833	Zhou *et al*., 2014
A1 (1.1)	HCVCAD22/14	Contaminant	2008	India	U606028	Desai *et al*., unpublished
A1 (1.1)	CSFV-PK15C-NG79-11	Contaminant	2011	India	KC503764	Tomar *et al*., 2015
A2	HEN03	Cattle	2012	China	KC176778	Zhang *et al*., unpublished
A2 (2.3)	Alfort/Tübingen	Pig		France	J04358	Meyer *et al*., 1989
A2 (2.1)	SKCDK	Pig	2009	China	GQ923951	Li *et al*., unpublished
A2 (2.1g)	GD19/2011	Pig	2011	China	KU504339	Gong *et al*., 2016
A2 (2.1)	HNLY-2011	Pig	2011	China	JX262391	Jiang *et al*., 2013
A2 (2.1)	HNSD-2012	Pig	2012	China	JX218094	Jiang *et al*., 2013
A2	S112	Contaminant	2014	China	MK118725	Zhang *et al*., 2014
A2	S173	Contaminant	2014	China	KF006975	Zhang *et al*., 2014
A5	IND/AS/GHY/G4	Pig	2014	India	KM362426	Ahuja *et al*., 2015
A5	CS/ML/911/IDP/13	Cattle	2013	India	KY860532	Ahuja *et al*., 2015
A5	CS/ML/AF/Umiam/14	Cattle	2014	India	KY860531	Chakraborty *et al*., 2018
B	5440/99	Sheep	1999	Spain	AY159514	Hurtado *et al*., 2003
D	S171	Contaminant	2014	China	KF006974	Zhang et al., 2014
A1	SWH	Pig	2004	China	DQ127910	Li *et al*., 2006

Conclusions

With reference to the current knowledge on CSFV, the evidence of circulation of CSFV in cattle by detection of 5'-UTR sequences in different regions from India and China was atypical. Nevertheless, the further observation of the sequencing of the entire E2 genomic region of CSFV from two sample isolates of bovine origin in northern India (Chakraborty *et al*., 2018) strongly corroborated the previous findings obtained in southern India and China. Furthermore, also in Kenya, where CSFV is endemic, a recent study provided serological and antigenic results demonstrating a similar circulation of CSFV in cattle (Muasya *et al*., 2022). This indicates that CSFV has the potential to cross species barrier, as other *Pestivirus* species, and it may represent an emerging health risk for the bovine species. In CSFV endemic environment, in order to avoid the spillover of the virus from suids to bovines, preventive measures should be considered. In this context, a biosecurity issue may be the rearing together of bovines with pigs. The farming promiscuity and the use of common pastures, if pigs are allowed to free ranging, represent risk factors to be controlled. Especially when CSFV live attenuated vaccines are administered, particular attention should be paid to hygienic measures and good heard management, including the application of biosecurity.

These findings are new and relevant for countries where CSFV control and eradication strategies are applied. Future studies will be necessary to further explore and confirm the adaptation and diffusion of CSFV in the bovine population.

It will be important to investigate on the genetic characteristics of field strains considering other genomic regions and possibly the full-length genome by internationally recognized tests, and to clarify differential diagnosis in case of clinical suspicions, in order to comply with the obligation to notify the disease, according to national norms of international reference rules as provided by the of the World Organization for Animal Health.

The present article was prepared on the base of a re-elaborated version of the study undertaken by Giangaspero and Zhang published in Open Veterinary Journal (Giangaspero and Zhang, 2020).

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