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Sequencing of E^{rns} Gene of a Live Attenuated Classical Swine Fever Cell Culture Vaccine Virus and its Comparison with Back Passages and Other CSFV Strains

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A B S T R A C T

The E^{rns} gene (681 bases) of a live attenuated classical swine fever (CSF) cell culture Indian vaccine virus (IVRI-CSF-BS)was sequenced and had only three nucleotide changes compared to its parental virulent virus at passage 6 in cell culture. The vaccine virus had Thymine at 151 and Guanine at 184 and 638 positions instead of Cytosine and Adenine at respective places in p6 virus. Out of these three mutations, nucleotide changes at 184 and 638 positions resulted in amino acid changes from Lysine to Glutamic acid and Arginine, respectively. The E^{rns} sequences of the vaccine virus were same as in the back passages up to passage 20. Further down at passage 15, the sequences were same except for Adenine at the 638 position, like it was in the p6 virus. Overall p15 had one amino acid change (Glutamic acid) and from p20 onwards, the viruses had two amino acid changes (Glutamic acid and Arginine). These changes were however not linked to virus attenuation, as the p20 virus produced fatal CSF infection in susceptible piglets. Additionally, the vaccine virus was phylogenetically more related to other CSF cell culture vaccine viruses derived from virulent CSF viruses than the lapinized vaccine viruses.

Introduction

Classical swine fever (CSF) is a highly fatal disease of pigs caused by *classical swine fever virus* belonging to the genus *Pestivirus* that also includes *bovine viral diarrhea virus* (BVDV) and *border disease virus* (BDV). It is a small enveloped virus with a single-stranded positive sense RNA of size of 12.3-kb with the genome structure of 5'UTR-Npro-C-E^{rns}-E1-E2-P7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3'UTR (Rice, 1996). Among the 11-12 viral proteins, E2 is the major glycoprotein anchored in the envelope and consists of 373 amino acids having molecular weight of 51-55 kDa. The E^{rns} is the second protein of importance, consisting of 227 amino acids (residue 268 to 494) with molecular weight of 41-44 kDaand is located at nucleotide positions 1178 to 1858of the genome (681 bases) downstream of the C protein (Zhang *et al.*, 2011, Leifer *et al.*, 2010). The E^{rns} protein isloosely attached to the envelope and has been known to be

responsible for virulence of the CSF viruses. Mutations at certain locations of the E^{rns} gene have been attributed for attenuation of the virus (Meyers *et al.,* 1999; Sainz *et al.,* 2008; Tews *et al.,* 2009). Since we have both a CSF cell culture vaccine virus (IVRI-CSF-BS) as well as its parental virulent virus in our laboratory, we investigated the E^{rns} genes of these viruses as well as some passages in between, for any evidence of change of sequences and its effect on virus attenuation. Phylogenetic relationship of the CSF cell culture vaccine virus has also been established with other known CSF reference vaccine viruses, lapinized as well as cell culture adapted strains.

Materials and Methods

Virus

A live attenuated CSF cell culture Indian vaccine virus (IVRI-CSF-BS) and some of its back passages such as passage numbers 15, 20, 33, 42, and 51 available in the laboratory were used for RNA extraction, PCR amplification and Sanger sequencing of the E^{rns} gene.

Primers

Primers were designed based on the available sequence of cell culture adapted CSF challenge virus at passage 6 (Accession No.MG599478) and other CSF viruses. Oligo Analyzer software was used for primer designing and were verified by Primer Blast (Table 1).

RNA isolation

Isolation of RNA from the live attenuated CSF vaccine virus (IVRI-CSF-BS) and its back passages were done directly from the virus samples, stored either in freeze dried or in liquid form at -20°C since 2017. Freeze dried virus samples were reconstituted directly in 1000 µl of Tri-reagent (Sigma Cat #T9424) and incubated at room temperature (RT) for 5 minutes. Two hundred µl of chloroform was added and incubated at RT for 15 min and centrifuged at 12000 rpm for 15 min at 4ºC. Aqueous phase was collected and incubated with equal volume of isopropanol at RT for 10 min and centrifuged at 12000 rpm for 10 min at 4ºC. Supernatant was discarded and the pellet was washed in 500 µl of 70% ethanol by vortexing for 8 seconds. The solution was then centrifuged at 7500 rpm for 5 min at 4ºC. Finally, the supernatant was discarded and the pellet was air dried for 10 minutes. The RNA pellet was dissolved in 11 µl of nuclease free water (Thermo Scientific Cat#R0582) containing 20 units of RNase inhibitor (Ribolock, ThermoScientific Cat # R0582) and stored at -80ºCuntil used.

For RNA extraction from the liquid viruses, 750 µl of TRI-reagent was added to 250 µl of the liquid viruses and thereafter followed the same steps as done for freeze dried viruses.

Reverse Transcription-PCR

The RNAs were reverse transcribed to synthesize 20µl of complementary DNA (cDNA) using a commercial kit (Thermo Scientific Revertaid First Strand cDNA synthesis kit, Cat # K1632) as per the manufacturers protocol and were stored at -20ºC until used.

Since the virus samples were almost two years old (stored since 2017), the cDNAs derived from these were first checked by a Taq polymerase PCR for amplification of the E^{rns} gene (763bp), before actually amplifying it using a proof reading KOD polymerase (Merck Cat #71842)for sequencing purpose. Briefly, 2.5µl cDNAs were added to the PCR reaction mix containing 10 pmol of forward

and reverse primers (CSFV-10-E^{rns} and CSFV-E^{rns}-34) (Table 1), 0.25units of Taq Polymerase (ThermoScientific Cat#EP0404), 1.5mM $MgCl_2$, 2mM dNTPs, in 1x Taq polymerase buffer and total reaction volume was made to 25 µl with nuclease free water. The PCR reactions were done in Eppendorf Master Cycler PCR machine. The reactions were subjected to initial denaturation of the cDNAs at 95ºC for 5 min, followed by 34 cycles of denaturation, annealing and extension at 95ºC for 30 sec, 48ºC for 30 sec and 72ºC, 60 sec respectively, followed by final extension at 72ºC for 5 minutes. Ten µl of the PCR reactions were mixed in 2µl of 6x loading dye and run in a freshly prepared 1.2% agarose gel in 0.5xTBE buffer containing 2µl Red safe dye (Intron Cat # 21141) for 45 min at 100V power and checked for 763 bp size amplicon under U.V. Transilluminator (Gel Doc XR,BioRad).

Once the cDNAs were checked, these were used for PCR amplification of the E^{rns} gene using a KOD hot start master mix, that contains a proof reading polymerase. Briefly, 2µl cDNAs were added to 50µl reaction mix containing 20 pmol of each of the primers, 1x KOD master mix and the final volume is made with nuclease free water. The PCR steps consisted of denaturation at 95ºC for 2 min followed by 30 cycles of denaturation, annealing and extension at 95ºCx20 sec, 48ºCx10 sec and 70ºCx15 sec, respectively and final extension at 70ºC for 10 seconds. The PCR products were detected in 1.2% agarose gel and further purified by gel extraction using a gel extraction kit (GCC Biotech Cat# G4628A).

Sequencing of the E^{rns} gene of the back passages

The 763bp of the E^{rns} amplicon of the CSF vaccine virus and its back passages (passage numbers 15, 20, 33, 42 and 51)(Fig. 1) were sequenced by Sanger's sequencing (Eurofins India Pvt ltd)using internal primers CSFV-E^{rns}-72and CSFV-E^{rns}-380 (Table 1).The sequences of only the 681 bp of the E^{rns} genes were aligned with the other CSFV sequences in NCBI blast analysis using MEGA X software. Phylogenetic analysis of the sequences was done by CLUSTAL W program.

Results and Discussion

Sequencing of E^{rns} gene of CSF cell culture vaccine and its back passages

The 681bp nucleotide sequence of the E^{rns} gene of the live attenuated CSF cell culture vaccine of Indian origin (IVRI-CSF-BS) has been submitted in GenBank (Accession number MT424777). Upon analysis of the sequence, we observed only three nucleotide changes in the E^{rns} gene in the vaccine virus compared to its parental virulent virus (Badasara *et al.*, 2017) at passage 6 in cell culture. The three mutations were Cytosine→Thymine at 151 and Adenine →Guanine at 184 and 638 positions. The first two nucleotide substitutions of C→T and A→ Gat 151 and 184 positions respectively were detected from passage 15 (Fig. 2a) and the third mutation of A→G at 638 position was detected from passage 20 onwards (Fig. 2b). The mutation of C→Tat 151 position was a synonymous mutation without any change in amino acid sequence, and this has been observed only in the Indian vaccine virus (IVRI-CSF-BS) compared to other CSF vaccine of virulent viruses including the parental virulent virus at passage 6 (Fig. 3).The second nucleotide substitution from A→G at position184 in the same passage 15 virus resulted in change of amino acid from Lysine to Glutamic acid at amino acid position 62. Subsequently, in passage 20 onwards, another similar nucleotide substitution from A→G was again observed at

638 position of the E^{rns} gene and this resulted in a change of amino acid from Lysine to Arginine at 213 position of E^{rns} protein. No further changes in the E^{rns} gene were observed in subsequent passages. Thus, the E^{rns} of the CSF virus at passage15 had only one amino acid change and from passage 20 onwards till the vaccine stage, the viruses had two amino acid changes. Our findings are in agreement in respect to a PK-15 adapted French CSF Thiverval vaccine strain (accession no. EU490425) with its parental virulent Alfort 187 strain, where also only three nucleotide changes were observed between the two viruses at 348, 638 and 669 positions with only one amino acid change from Lysine-to-Arginine at 213 position of E^{rns} protein (Fan *et al.*, 2008). Similarly, while comparing the E^{rns} sequences of a guinea pig adapted GPE- vaccine strain (Accession no. D49533) with its ancestral virulent ALD strain, six mutations were observed at 187, 284, 318, 320, 321 and 435 positions with only three amino acid changes from Glycine-to-Arginine at 63 position, Asparagine-to-Serine at 95 position and Alanine-to-Aspartic acid at 107 amino acid positions in E^{rns} protein (Ishikawa *et al.*, 1995). Thus, it may be possible that the mutations as accumulated in the E^{rns} gene, actually depend on the type of the cell culture used for passaging of the virulent parental virus.

Table.1 Primers used in the study

S.no	Primer name	Sequence 5'- 3'	Genome position	Mer	Purpose
1	CSFV-10-E^{rns}-F	gcaattatgttrtaccaacc	574-593 in the C protein gene upstream of the E^{rns} gene	20	For PCR amplification of the E^{rns} gene (product size of 763 bp)
	CSFV-E^{rns}-34-R	rttagtgtaccatatgtacc	1337-1318 in the E1 protein gene downstream of the E^{rns} gene	20	
2	CSFV-E^{rns}-72F	gtcagcagaagtttgcatg	676-694 in E^{rns}	19	For use as internal primers for sequencing
	CSFV-E^{rns}-380R	cctgagtgaccacattgac	985-967 in E^{rns}	19	

Fig.1 PCR amplification of E^{rns} gene of CSFV vaccine virus and its back passages. A 763 bp amplicon was generated for the back passages such as P15, P20, P33, P42, P51 (Lanes 1, 2, 3, 4 and 5 respectively) and the CSF vaccine virus (Lane 6); M – 100bp DNA ladder; Lane 7- No amplification in the control. The 763 bp amplicon was sequenced and only 681 bp sequences of the complete E^{rns} gene was used in sequence analysis

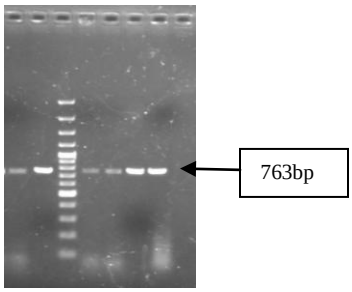


Fig.2a Alignment of 142 -191 positions of E^{rns} genes of CSF cell culture vaccine virus (Sl. No. 7) with its back passages (Sl. No. 2 – 6) and the parental virulent virus at P-6 in cell culture (Sl. No. 1) are shown. Two mutations of C151T and A184G were observed from P15 onwards

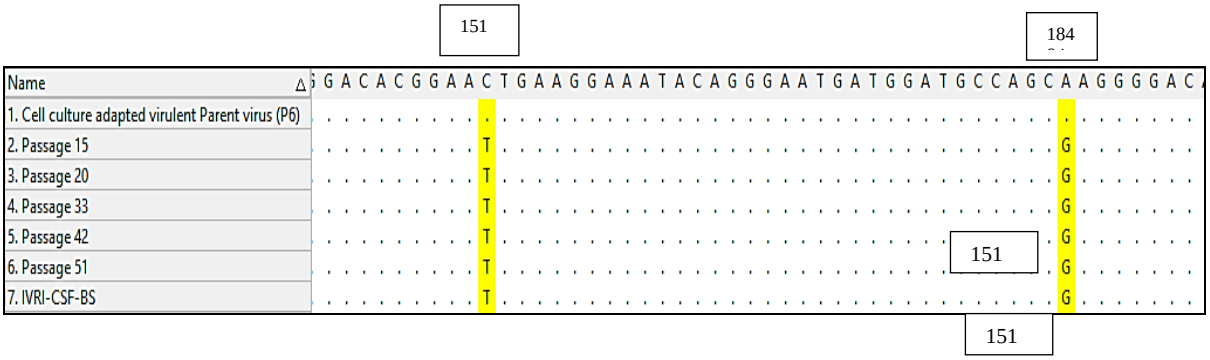


Fig.2b Alignment of 604 -644 positions of E^{rns} genes of CSF cell culture vaccine virus (Sl. No. 7) with its back passages (Sl. No. 2 – 6) and the parental virulent virus at P-6 in cell culture (Sl. No. 1) are shown. Only one mutation of A638G was observed from P20 onwards. *[Nucleotides of the E^{rns} of the CSF vaccine virus other than at the positions 151,184 and 638 were similar to that of the parental virulent virus at p6 in cell culture]*

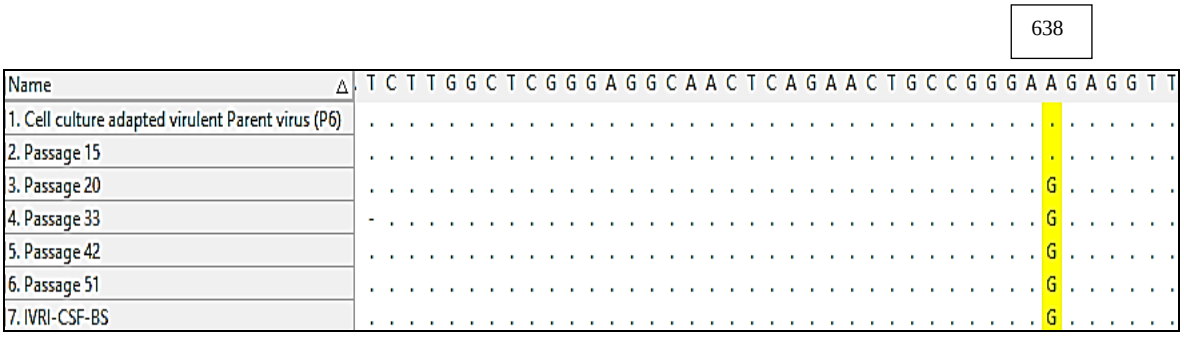


Fig.3 The nucleotide Thymine (T) at 151 position of E^{rns} gene (1-681 nucleotides) is unique to the CSF vaccine virus (IVRI-CSF-BS) compared to its parental virulent virus at passage 6 in cell culture (Serial No. 2) and other 23 CSF vaccine or virulent field viruses which had Cytosine (C) in the same position.

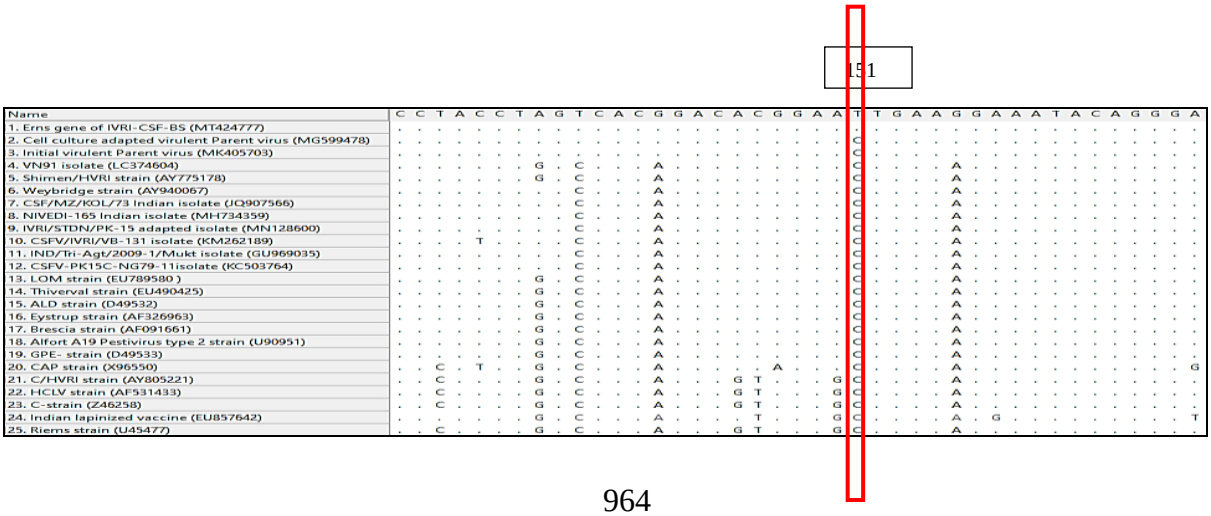


Fig.4 Percent Identity Table based on the E^{rns} nucleotide sequences of CSF viruses showing the CSF cell culture vaccine virus (IVRI-CSF-BS) has 92.8 to 94.9% homology with other cell culture vaccines whereas 90 to 91.5% with the Lapinized vaccines and 81.4 to 94.9% with the field isolates.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1		90.0	91.5	93.7	92.8	94.3	93.8	81.4	82.1	82.2	81.9	93.7	90.0	93.7	91.3	90.0	94.9	94.9	81.9	93.8	93.8	81.9	91.3	1
2	11.0		92.8	94.5	94.3	47.2	53.8	49.2	49.2	48.8	48.8	45.1	99.4	94.4	92.8	98.8	45.4	44.8	84.6	94.0	93.4	84.1	44.4	2
3	9.3	7.6		89.0	71.1	36.2	41.0	35.9	36.0	35.8	35.7	34.3	69.5	71.2	74.4	69.6	34.7	34.2	83.4	92.3	82.8	83.2	35.1	3
4	6.7	5.8	5.3		79.1	39.4	44.8	39.2	39.3	39.1	38.9	38.3	75.3	79.3	76.2	75.7	37.9	37.4	84.6	96.6	90.1	84.3	36.5	4
5	7.7	5.9	5.4	1.2		49.0	55.8	49.2	49.4	49.2	49.0	47.4	94.0	98.8	95.1	94.2	47.1	46.5	84.9	96.5	96.0	84.4	45.4	5
6	6.0	8.0	5.6	3.6	4.1		99.0	83.1	83.4	83.0	83.0	89.7	92.5	96.5	94.6	92.4	92.1	91.4	83.1	98.9	98.9	82.9	86.9	6
7	6.5	8.0	6.2	3.8	4.1	1.0		83.9	84.2	84.0	83.9	79.8	92.5	96.4	94.3	92.4	81.8	80.8	83.9	98.6	98.4	83.2	77.5	7
8	22.4	18.0	21.0	18.5	17.8	19.8	18.7		97.1	96.7	96.3	69.1	84.5	83.8	82.6	84.0	69.5	68.5	96.8	83.2	83.1	96.2	67.1	8
9	21.3	17.9	20.7	18.1	17.5	19.4	18.2	2.9		97.7	97.7	69.3	84.6	84.1	82.8	84.1	69.6	68.6	99.6	83.8	83.4	97.3	67.4	9
10	21.1	18.7	21.2	18.5	17.8	20.0	18.6	3.3	2.2		97.4	69.1	84.0	83.9	82.4	83.5	69.5	68.6	97.5	83.3	83.2	96.7	67.1	10
11	21.6	18.7	21.8	19.0	18.3	20.0	18.7	3.7	2.2	2.7		68.8	84.0	83.5	82.1	83.5	69.3	68.3	97.5	83.2	83.0	96.4	66.9	11
12	6.7	6.3	4.8	0.3	1.2	3.3	3.4	19.0	18.6	19.2	19.6		94.1	99.9	95.5	94.1	97.8	97.0	83.9	97.0	96.4	84.1	94.6	12
13	11.0	0.1	7.5	5.7	5.9	8.0	8.0	18.0	17.9	18.7	18.7	6.3		94.2	92.6	98.4	45.4	44.8	84.4	93.6	93.1	83.9	44.4	13
14	6.7	5.8	5.3	0.8	1.2	3.6	3.7	18.9	18.4	18.8	19.3	0.1	5.7		95.2	94.4	47.3	46.7	84.4	96.6	96.0	83.9	45.6	14
15	9.4	7.6	0.8	5.0	5.2	5.7	6.0	20.6	20.3	20.8	21.3	4.7	7.4	5.0		92.6	46.0	45.5	83.7	94.4	94.4	83.3	46.5	15
16	11.0	0.9	7.6	5.6	5.7	8.1	8.2	18.7	18.5	19.4	19.4	6.3	0.8	5.5	7.5		45.4	44.8	84.3	94.1	93.5	83.8	44.4	16
17	5.4	6.3	4.5	2.0	2.6	1.5	1.6	19.2	19.0	19.2	19.6	2.1	6.3	2.2	4.9	6.3		98.4	83.6	98.4	98.1	83.9	94.1	17
18	5.4	7.8	6.0	3.4	4.0	2.2	2.9	20.7	20.5	20.7	21.1	3.0	7.8	3.5	6.2	7.8	1.6		82.5	97.1	96.8	82.8	93.1	18
19	21.6	17.9	19.6	17.9	17.4	19.8	18.7	3.3	0.4	2.6	2.6	18.8	17.6	18.1	19.1	18.3	19.2	20.7		80.8	71.9	95.0	28.8	19
20	6.5	6.3	6.1	3.5	3.6	1.1	1.5	19.7	18.9	19.6	19.7	3.1	6.3	3.5	5.8	6.2	1.6	2.9	18.5		89.0	83.8	34.5	20
21	6.5	7.0	6.3	4.2	4.2	1.1	1.6	19.9	19.3	19.7	19.9	3.7	6.9	4.1	5.9	6.8	1.9	3.2	19.4	0.9		83.3	38.3	21
22	21.6	18.6	19.6	18.3	18.2	20.2	19.8	4.0	2.8	3.4	3.7	18.6	18.4	18.9	19.7	19.0	18.8	20.3	2.8	18.9	19.7		29.5	22
23	9.4	8.5	3.1	5.6	6.1	6.5	6.9	23.1	22.5	23.1	23.6	5.6	8.5	5.8	3.6	8.5	5.3	6.4	22.7	6.7	7.2	22.5		23
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	

1 CSFV IVRI BS .seq
2 CSF KC strain Russia (AF099102).seq
3 CSFV C strain China (AY805221).seq
4 CSFV Thiverval strain (D49533).seq
5 CSFV GPE- strain Japan (D49533).seq
6 CSFV IND MZ (JQ907566).seq
7 CSFV IND Tri (GU969035).seq
8 CSFV IND UK DDN (KC534849).seq
9 CSFV IND UK LAL (KC534848).seq
10 CSFV IND UP-Izt (GU969033).seq
11 CSFV INDBih (GU969034).seq
12 CSFV Korea (MN558884).seq
13 CSFV lapinised strain Russia (KM522833
14 CSFV LOM strain Rep. of Korea (EU789580
15 CSFV Riems strain Germany (U45477) .seq
16 CSFV ROVAC Sweden (KJ873238) .seq
17 CSFV shimen strain China (AY77517).seq
18 CSFV Weybridge UK (AY940067).seq
19 CSFV_IND-UK-LAL.seq
20 CSFV-IND_PK15C-NG79 (KC503764).seq
21 CSFV-IVRI-VB-131 (KM262189).seq
22 CSFV-UP-GZ-NVD-11 (JQ861548).seq
23 Lapinised vaccine IVRI India (EU857642)

Fig.5 Phylogenetic analysis of CSF vaccines based on E^{rns} gene sequences. The Indian cell culture vaccine virus (IVRI-CSF-BS) is in the same cluster along with its parental virulent virus at passage 6 in cell culture (MG599478) and the original Indian virulent virus (MK405703.1) from which the vaccine virus was derived. These viruses are more closely related to the other cell cultures vaccines than the lapinized vaccines.

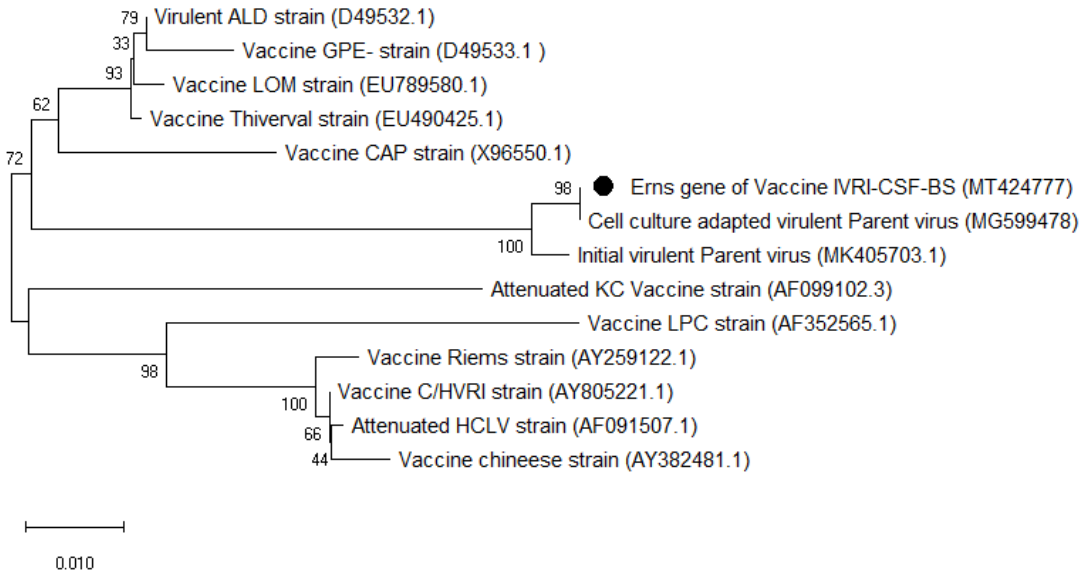


Fig.6 Rectal temperature of pigs inoculated with P20 virus. Biphasic temperature reactions were observed in both the pigs (916 and 919) with peak temperature 104.5° F from 7 to 11 days post inoculation. Both the pigs had died of CSF infection after showing the clinical symptoms of CSF and had marked leucopenia (1400 and 2950 cells/cu. mm). The passage 20 virus was a hot virus although two amino acid changes have been accumulated in the E^{rns} sequence compared to its parental virulent virus.

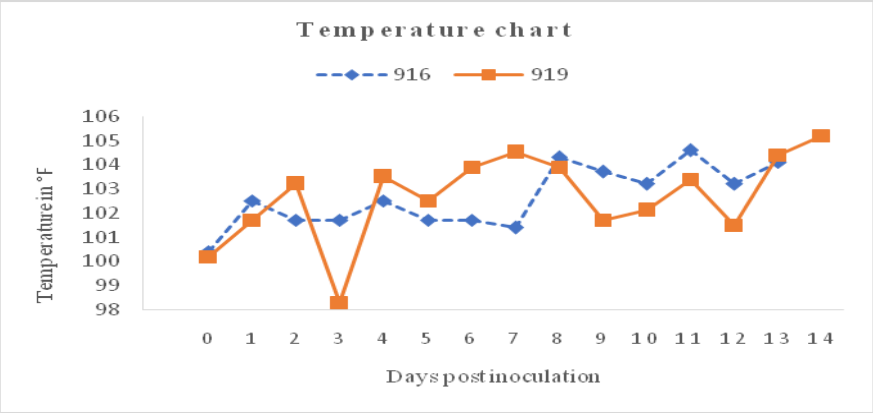
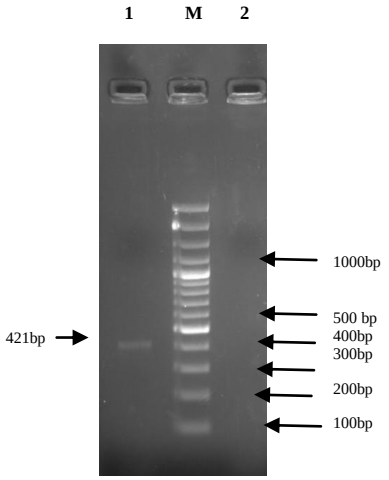


Fig.7 Detection of CSFV genome by RT-PCR in the pig blood collected at viraemia stage (10 dpi) after inoculation of the P20 virus**.** A 421 bp amplicon was detected in the blood of pig no. 919 (Lane-1); M-100 bp DNA ladder; Lane 2- No amplification in the negative control



CSFV E^{rns} gene based phylogenetic analysis with CSFV reference vaccine strains

The live attenuated CSF cell culture Indian vaccine virus (IVRI-CSF-BS) has been developed recently and its molecular characterization has not yet done. We compared the E^{rns} gene of the Indian cell culture vaccine virus with that of the lapinized vaccines (such as Chinese C strain, Riems strain, Russian KC strain, Indian Lapinized strain, Swedish ROVAC strain, etc) as well as other cell culture vaccines derived from virulent field isolates (such as Thiverval strain, GPE^{-} strain, LOM strain, etc) (Fig. 4).

Based on the sequence analysis of the E^{rns} genes of these viruses, we observed that our cell culture Indian vaccine virus has more sequence homology (92.8 to 94.9%) with other live attenuated cell culture vaccines than the lapinized vaccines (90 to 91.5%) including the widely known Chinese C strain (91.5%) and the Indian lapinized vaccine (91.3%). Phylogenetic analysis of the E^{rns} genes also revealed the same. Our vaccine virus (IVRI-CSF-BS) is more closely related to other CSF cell culture adapted viruses than the lapinized vaccine strains (Fig 5).Additionally, the CSF Indian vaccine virus (IVRI-CSF-BS) and its parental virulent virus have been found to stand out as a separate group within the CSF cell culture vaccine viruses.

Correlation of mutations in the E^{rns} gene of CSFV with virus attenuation

The nucleotide sequences of the E^{rns} genes of the Indian vaccine virus and the back passages revealed that the virus had two changes in amino acid of the E^{rns} protein from passage 20 onwards. Since E^{rns} in known to be a virulence determinant of CSF viruses and mutations in this gene is known to cause virus attenuation (Meyers *et al.,* 1999; Sainz *et al.,* 2008; Tews *et al.,* 2009), we attempted to look for the virus attenuation of the passage 20 virus *in vivo*. However, we observed that these amino acid changes in the E^{rns} are not linked to virus attenuation as the 20^{th} passage virus at a dose of $10^{5.5}$ $TCID_{50}$produced CSF infections in two susceptible piglets which ultimately succumbed to the infection in 17 days. The piglets had the CSF symptoms starting from anorexia, depression to fever up to 104.5°F (Fig. 6), skin rashes in the extremities, emaciation, paralysis of hindquarters, respiratory distress and diarrhea before finally dying due to the disease. Both the animals had marked leukopenia such as 1400 cells/cu.mm in one and 2950 cell/cu.mm in the second one, which is most pathognomic for CSF infection. Postmortem examination of these animals were also suggestive of CSF infection such as pinpoint hemorrhages on the kidneys (turkey egg appearance), hemorrhages on the tonsils, necrotic lesions in the intestine and necrosis of mesenteric and other lymph glands (Badasara *et al.,* 2017). The blood collected at viraemia stage were also positive in CSF specific PCR (Fig. 7). Thus, we confirmed that the passage 20 virus was still a hot virus and the two amino acid changes had actually no effect on the virus attenuation *invivo*. Our study is in agreement with an earlier study on the virulent Brescia strain, in which a Serine-to-Arginine at 209 position did not reduce virulence in pigs (Van Gennip *et al.,* 2004) although this position is known to be responsible for heparin sulfate receptor binding into the cells (Hulst *et al.,* 2000).

In conclusion there was not much change in the E^{rns} gene between the virulent and the vaccine virus. The unique change *i.e.,* C→T at 151 position can be used to identify the virus as it is observed only in the Indian vaccine virus (IVRI-CSF-BS) and can be attributed as a signature nucleotide change. The little changes occurred were however not related to virus attenuation.

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References

Badasara, S.K., Mohan, M., Sah, V., Kumari, P., Upamanyu, V., Dhar, P., Tiwari, A.K., Chander, V. and Gupta, V.K. (2017). Replacement of Animal Model for Propagation of Classical Swine Fever Challenge Virus by Adaption in the PK-15 Cell Line. *Journal of Animal Research*, 7(3): 581-586.

Fan, Y., Zhao, Q., Zhao, Y., Wang, Q., Ning, Y. and Zhang, Z. (2008). Complete genome sequence of attenuated low-temperature Thiverval strain of classical swine fever virus. *Virus genes*, 36(3): 531-538.

Hulst, M.M., Van Gennip, H.G.P. and Moormann, R.J.M., (2000). Passage of classical swine fever virus in cultured swine kidney cells selects virus variants that bind to heparan sulfate due to a single amino acid change in envelope protein E^{rns}. *Journal of virology*, 74(20): 9553-9561.

Ishikawa, K., Nagai, H., Katayama, K., Tsutsui, M., Tanabayashi, K., Takeuchi, K., Hishiyama, M., Saitoh, A., Takagi, M., Gotoh, K. and Muramatsu, M. (1995). Comparison of the entire nucleotide and deduced amino acid sequences of the attenuated hog cholera vaccine strain GPE− and the wild-type parental strain ALD. *Archives of Virology*, 140(8): 1385-1391.

Leifer, I., Hoffmann, B., Hoper, D., Rasmussen, T.B., Blome, S., Strebelow, G., Horeth-Bontgen, D., Staubach, C. and Beer, M. (2010). Molecular epidemiology of current classical swine fever virus isolates of wild boar in Germany. *Journal of General Virology*, 91(11): 2687-2697.

Meyers, G., Saalmüller, A. and Büttner, M. (1999). Mutations abrogating the RNase activity in glycoprotein Erns of the pestivirus classical swine fever virus lead to virus attenuation. *Journal of Virology*, 73(12): 10224-10235.

Rice, C.M. (1996). Flaviviridae: the viruses and their replication, In: Knipe, B.N. F.D.M., Howley, P. (Eds.), Fundamental Virology, Third ed. Lippincott Raven, Philadelphia: 931–959.

Sainz, I.F., Holinka, L.G., Lu, Z., Risatti, G.R. and Borca, M.V. (2008). Removal of a N-linked glycosylation site of classical swine fever virus strain Brescia Erns glycoprotein affects virulence in swine. *Virology*, 370(1): 122-129.

Tews, B.A., Schürmann, E.M. and Meyers, G. (2009). Mutation of cysteine 171 of pestivirus Erns RNase prevents homodimer formation and leads to attenuation of classical swine fever virus. *Journal of Virology*, 83(10): 4823-4834.

Van Gennip, H.G.P., Vlot, A.C., Hulst, M.M., De Smit, A.J. and Moormann, R.J.M., (2004). Determinants of virulence of classical swine fever virus strain Brescia. *Journal of Virology*, 78(16): 8812-8823.

Zhang H., Cao H.W., Wu Z.J. and Cui Y.D. (2011).A Review of Molecular Characterization of Classical Swine FeverVirus (CSFV). *Israel Journal of Veterinary Medicine*, 66 (3):89-95.

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