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Adaptation and propagation of classical swine fever virus in different cell lines

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ABSTRACT

Adaptation and propagation of 10 classical swine fever virus (CSFV) field isolates and the lapinized vaccine strain of CSFV were made in PK-15 and PS cell lines. None of the field isolates and the vaccine strain could be adapted in MDBK cell line. Four field isolates of CSFV produced cytopathic effect in both the cell lines. All the field isolates and the vaccine strain were propagated in both the cell lines up to fifth passage level after the initial adaptation. A gradual increase of the viral antigen based on the OD value in sandwich ELISA test was observed from the first to the fifth passages in the cell lines. The OD value of the virus isolates in the PK-15 cell line ranges from 0.612 to 1.538 and in the PS cell line ranges from 0.703 to 1.742 at fifth passage level. The log $TCID_{50}$ of the virus isolates in the PK-15 cell line varies from 2.75 to 6.25 and in the PS cell line ranges from 2.25 to 6.50 at the fifth passage level. Although no statistically significant differences in the OD values and the log$TCID_{50}$ values of the virus isolates and the vaccine virus strain was observed in the PK-15 and PS cell lines, but the use of PS cell line for adaptation and propagation of CSFV is the first report and this cell line appears to be one of the best alternatives of PK-15 cell line for propagation of CSFV.

Key words: CSFV, PK-15, PS, MDBK, Adaptation and propagation

Classical swine fever virus (CSFV) is a member of the genus *Pestivirus* and the family *Flaviviridae.* The virus is the causative agent of classical swine fever, the most feared and devastating disease of pigs. Classical swine fever has been reported from all the pig producing states of India and is considered to be the major constraint for the growth of piggery in the North Eastern Region (Sarma *et al*. 2008). Because of its significant impact on the pig husbandry, control of CSF in North Eastern Region of India in particular and the country as a whole, is the urgent necessity.

Lapinized swine fever virus vaccine has been used to prevent the disease in India, but the vaccine is inadequate and there are reports of occurrence of the disease in vaccinated pigs (Barman *et al*. 2003, Sarma 2007). The demand for CSF vaccine in India can be fulfilled by cell culture vaccine and development of a cell culture attenuated vaccine virus from local isolates will be more useful. Good growth of vaccine virus and easy attenuation of field isolates of CSFV are important for developing attenuated cell culture vaccine. Porcine cell line particularly the PK-15 cell line has normally been used to isolate and propagate the virus. There are also few reports about the propagation of CSFV in other cell lines like SK-6, MPK and RK-13 (Moorman and Hulst, 1988,Rivero *et al.,* 1988), but there is no report about the adaptation and propagation of CSFV in PS cell line, a kidney cell line of porcine origin and also other heterologous cell line like MDBK. Therefore, the present study was undertaken to adapt and propagate CSFV field isolates and the lapinized CSF vaccine virus strain in PS and MDBK cell lines and to compare with PK 15 cell line.

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MATERIALS AND METHODS

Cell lines: Three established cell lines, viz. PK-15, PS, and MDBK were used for adaptation and propagation of CSFV. The cell lines used in the study were obtained from the National Centre for Cell Science (NCCS), Ganeshkhind, Pune, India. The cells were maintained in the laboratory by continuous subculturing. The subculturing of cell monolayer was done as per the European Union Diagnostic Manual (Anonymous 2002).

CSFV isolates and vaccine virus: CSFV field isolates (10) from different places of Asom and the cell cultured adapted lapinized CSF vaccine virus strain were taken from the repository of National Fellow Laboratory, Department of Microbiology, College of Veterinary Science, AAU, Khanapara, Guwahati for the study.

Adaptation in PS and MDBK cell lines: All the virus isolates and the vaccine virus at fifth passage level in PK-15 cell line were used for adaptation in PS and MDBK cell lines.

The two cell lines were grown in 25 cm^2 tissue culture flask with Eagles minimum essential (EMEM) medium containing 10% foetal bovine serum (FBS). Confluent cell monolayers were inoculated with each of the virus isolates and the vaccine virus. The inoculated flasks were incubated at 37°C in a CO_2 incubator for 1 h for adsorption of the virus. After adsorption, the inoculum was discarded from the flasks and approximately 8ml of the maintenance medium (EMEM) containing 5 % FBS was added and the flasks were incubated at 37°C in a CO_2 incubator for 4 - 6 days. After the incubation period the flasks were stored at –20°C. The cell culture supernatant was harvested from the flasks by alternate freezing and thawing for 3– to 4– times and the cell suspension was clarified by centrifugation at 15 000 rpm for 10 min at 4°C. The supernatant collected was stored at –20°C for further study. Subsequent passages up to fifth of each of the virus isolates were given by transferring 1 ml of the cell supernatant to freshly prepared cell monolayer. After the fifth passages in both the cell lines, the harvested cell culture supernatants were examined for CSFV antigen by sandwich ELISA (Sarma and Sarma 1995).

Propagation in PK-15 and PS cell line: All the CSFV isolates and the vaccine virus strain at fifth passage level in PK-15 and PS cell lines were further given 5 more serial passages in the 2 cell lines. The release of the CSFV antigen in the cell culture supernatant after 3– to 4– times freezing and thawing and centrifugation was also determined by the sandwich ELISA (Sarma and Sarma 1995).

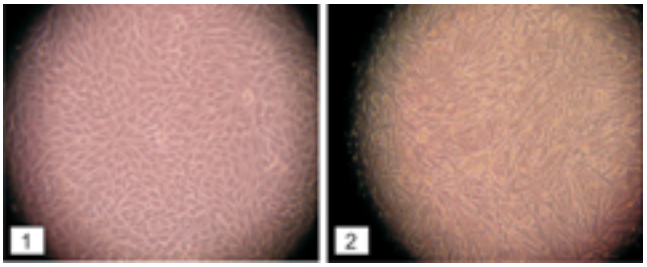
Determination of the virus titre: The titre of all the 10 CSFV isolates and the vaccine virus strain at the fifth passage level in the PK-15 and PS cell lines was determined by calculating $TCID_{50}$. Immunoperoxidase test (IPT) was performed as per European Union Diagnostic Manual (Anonymous 2002) with slight modification for calculation of $TCID_{50}$. For IPT the cell lines were grown in 96-well microtitre tissue culture plate and the plate wells containing confluent cell monolayers were used. Each of the virus isolates and the vaccine virus was diluted starting from 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} aseptically in sterile tissue culture microtitre plate. From each dilution of the virus 100 µl was transferred to 4 wells of the 96-well microtitre tissue culture plate containing confluent cell monolayer. The plate was then incubated for 3–4 days at humidified atmosphere containing 5% CO_2. After incubation the cell monolayer was rinsed briefly with wash buffer (0.15 M NaCl solution with 1 % Tween 20, pH 7.4) and fixed by incubating the plate at 70°C to 80°C for 2 h. The monolayer was then overlaid with the CSFV specific monoclonal antibody (Mab), diluted @ 1:20 in blocking buffer (0.5M NaCl solution containing 1 % Tween 20, pH 7.4) and incubated at 37°C in CO_2 incubator for 30 min. The plate was washed 5-times with the wash buffer after the incubation period and chicken anti-mouse IgG horse radish peroxidase conjugate diluted @1:500 in the blocking buffer was then added at a volume of 50 µl to each well of the microtitre plate. After incubation at 37°C for 30 min the plate was washed again and 50 µl of chromogen substrate solution (3- amino-9-ethyl carbazole and H_2O_2) was added to each well and allowed to react for 15 to 30 min at room temperature. Cell monolayers inoculated with CSFV negative cell culture fluid and normal cell culture fluid were kept as control throughout the test procedure. The monolayer was examined under inverted microscope and cytoplasm of infected cells having dark red colour was considered as positive. Logarithm of the highest virus dilution with the reaction rate(R) 1.00 was taken for calculation of $TCID_{50}$. The $TCID_{50}$ was calculated by using the formula of Karber (1931).

Statistical analysis: The data obtained from the present study were analysed statistically following the procedure described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

All the CSFV isolates and the lapinized CSF vaccine virus grown in PK-15 cell line at fifth passage level could be adapted in the PS cell line, but failed to adapt in MDBK cell line after giving 5 serial passages. Four of the CSFV isolates, which showed cytopathic effect (CPE) in the PK-15 cell line also showed the CPE in the PS cell line. The cytopathic effect was observed after 72 h of incubation. The CPE was characterized by rounding of cells, degeneration, elongation and detachment of the cells from the surface of the flasks. The vaccine virus did not show any CPE in PK-15 and PS cell lines (Figs 1-2).

All the CSFV isolates and the vaccine virus were propagated in PK-15 and PS cell lines and gradual increase in mean OD value from the first to fifth passages was observed. In PK-15 cell line, out of the 10 isolates 3 isolates showed consistently good growth in each passage level. Six isolates have shown moderate growth in each passage level and only 1 isolate showed poor growth up to fifth passage level. The vaccine virus has shown good growth in each passage level as indicated by the gradual increase of the OD value at different passage level. The ELISA OD value of the virus isolates ranges from 0.392 to 1.507 at first to fifth passage. The vaccine virus showed OD value 0.402 at first passage and OD value 1.370 at fifth passage level in the cell line.



Figs 1–2. **1.** Normal PK-15 cell monolayer (100X). **2.** PK-15 monolayer showing CPE (100×) after 72 h of CSF virus infection.

Table 1. Comparison of OD value of CSFV isolates and vaccine virus in PK-15 and PS cell lines at 5th passage level

Virus isolates Laboratory no.	Corrected OD value of the CSFV isolates in ELISA test at 5th passage level in	
	PK -15	PS
3030 T	1.174	1.044
3023 K	1.352	1.538
1062 K	0.931	1.186
1063 S	1.507	1.742
3052 K	0.928	0.809
3044 K	0.612	0.703
995 T	0.809	0.823
991 K	1.298	1.443
692 S	1.003	0.980
852 S	1.538	1.663
Vaccine virus	1.370	1.461

Table 2. Log TCID $_{50}$/ml of the CSFV isolates and vaccine virus detected by IPT at 5th passage level

Virus isolates Laboratory no.	Log $TCID_{50}$/ml at 5th passage level in	
	PK-15 cell line	PS cell line
3030 T	4.75	5.25
3023 K	5.50	6.25
1062 K	4.25	5.25
1063 S	5.75	6.25
3052 K	4.50	4.25
3044 K	2.75	2.25
995 T	3.25	2.75
991 K	5.50	5.75
692 S	5.25	4.50
852 S	6.25	6.50
Vaccine virus	5.50	5.75
Mean±Se	4.840±0.325	4.977±0.427

In PS cell line, out of the 10 CSFV isolates 4 isolates showed consistently good growth in each passage level. Five isolates showed moderate growth in each passage level. Only 1 isolate showed poor growth in each passage. The OD value of the CSFV isolates ranges from 0.394 to 1.742 at first to fifth passage and the OD value of the vaccine virus was 0.553 and 1.461 at first and fifth passage level, respectively, in the cell line. Comparison of the OD value of the CSFV isolates and the vaccine virus in PK-15 and PS cell lines at fifth passage level is shown in the Table 1.

Although increase of OD value of the CSFV isolates and the vaccine virus in the PS cell line was higher than the OD value in PK-15 cell line, the statistical analysis showed no significant difference between the OD value in both PK-15 and PS cell line ('t' value =0.426; P>0.05).

The $TCID_{50}$/ml of the 10 CSFV isolates and vaccine virus in PK-15 cell line at different passage level ranges from 1.25 to 6.25. There was gradual increase in mean log$TCID_{50}$ from the first to fifth passages. Out of the 10 CSFV isolates at fifth passage 1 isolate showed $TCID_{50}$/ml above 6.00, 4 isolates showed $TCID_{50}$/ml above 5.00, 3 isolates have shown $TCID_{50}$ above 4.00 and 2 CSFV isolates showed $TCID_{50}$ below 4.00.

The $TCID_{50}$/ml of the CSFV isolates in the PS cell line at different passage levels ranges from 1.25 to 6.50. A steady increase in mean log$TCID_{50}$ from the first to fifth passages was observed. Out of the 10 CSFV isolates at fifth passage 3 isolates showed $TCID_{50}$ above 6.00, three isolates showed $TCID_{50}$ above 5.00, two isolates has shown $TCID_{50}$ above 4.00 and 2 isolates showed $TCID_{50}$ below 3.00. The $TCID_{50}$/ml of the vaccine virus was above 5.00 at fifth passage level in both PK-15 and PS cell lines.

Comparison of the $TCID_{50}$/ml of the CSFV isolates and the vaccine virus at fifth passage level is given in the Table 2. Out of the 10 CSFV isolates at fifth passage level in PK-15 cell line only 1 isolate showed $TCID_{50}$ above 6.00, while 3 isolates at fifth passage level in PS cell line showed $TCID_{50}$ above 6.00. Similarly the vaccine virus has shown 5.50 $TCID_{50}$/ml in PK-15 cell line and 5.75 $TCID_{50}$/ml in PS cell line. However, no significant difference was found between the log $TCID_{50}$/ml of the CSFV isolates and the vaccine virus in PK-15 and PS cell line ('t' value =0.309; P>0.05).

In the present study, 10 CSFV field isolates and vaccine virus strain were successfully adapted and propagated in 2 porcine kidney cell lines PK-15 and PS. Eagle's minimum essential medium (EMEM) containing 10 % fetal calf serum (FCS) supported the growth of both PK-15 and PS cells. Although most of the CSFV isolates are non cytopathic in nature, but in the present study a few of the CSFV isolates were cytopathic in the 2 cell lines. Isolation of cytopathic CSFV in PK-15 cell line from field outbreak was also reported (Sarma *et al.* 2008). Use of PK-15 cell line for isolation and propagation of CSF virus field isolates was reported by various workers (Kaden *et al*.1999, Sarma *et al.* 2007). Dhar *et al.* (2008) have reported the adaptation of the lapinized swine fever virus in PK-15 cells from the third passage onwards. The PS cell line was used for the first time for adaptation and propagation of CSFV in the present study and the findings suggested that PS cell line is the best alternative of PK-15 cell line for propagation of CSFV. In the present study none of the CSFV field isolates and the lapinized vaccine virus could be adapted in MDBK cell line. The MDBK cell line is used for isolation of pestivirus particularly bovine viral diarrhoea virus (BVDV), but in the present study none of the CSFV isolates and the vaccine virus could be adapted in the cell line. This finding suggested that CSFV though share antigenic epitopes with BVDV, but the cell tropism of CSFV appears to be distinct. However, Pinyochon (2003) reported the adaptation and propagation of classical swine fever virus lapinized Chinese strain in SK-6 and bovine foetal muscle (BFM) cell lines. This indicated that the cell tropism of lapinized strain of CSFV may be

modified due to serial passages in rabbit and this facilitate the growth of the virus strain in porcine as well as in other cell lines like BFM.

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