



Preliminary studies on selection of a vaccine candidate of Classical Swine Fever Virus

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Classical swine fever (CSF) is also known as hog cholera and affects both domestic pigs and wild boars. CSF, a highly contagious, economically important viral disease is caused by a Pestivirus of the family Flaviviridae. The severity of the disease depends on the age of the pigs, virulence of the virus and time of infection. As far as the virulence of the virus is concerned, low and moderately virulent isolates cause a chronic and mild infection. More virulent viruses cause pyrexia, leucopenia, haemorrhagic lesions and death (Rout and Saikumar 2012). Mortality due to CSF is highly variable and could range from 8.2-45% (Kumar *et al.* 2007, Manoharan *et al.* 2011). The morbidity and mortality can still be higher in unvaccinated young pigs of less than 3 months age (Rathnapraba *et al.* 2012). Vaccination is the only option available for the control of this very important disease of swine.

In India, though the farm or home grown pigs are vaccinated against CSF, free ranging desi or country pigs are generally not vaccinated. This results in the all-time existence of susceptible pig populations. The lapinized CSF Virus (CSFV) vaccine used in India is not freely available and there are still some concerns about the fool-proof efficacy of the vaccine. Use of *in vitro* cultured cells for the production of viral vaccines is always preferred since it could avoid the use of animals for vaccine production. PK-15 cells are the most preferred cells for the propagation and adaptation of CSFV, though no visible cytopathic effects (CPE) are produced by CSFV in PK-15 cells (Chander *et al.* 2014, Gonzalez *et al.* 2015). In an attempt to develop a cell culture based CSFV vaccine employing an Indian isolate, the adaptability of two field isolates and one lapinized strain of CSFV in PK-15 cells to evolve a vaccine candidate was studied.

Cells and CSFV isolates: PK-15 cell line procured from ATCC, USA and maintained at the Department of Animal

Biotechnology, Madras Veterinary College, Chennai was used for the propagation and adaptation of the field isolates of CSFV. PK-15 cell line was maintained and sub-cultured using standard cell culture procedures. Two CSFV isolates (CSFV-Ind-AP/Tru-09-002–TS1 and CSFV-Ind-TN/Veng-09-020-Vn) recovered from field outbreaks and a lapinized CSFV (CSFV Lapinized Virus - M) were used for adaptation. CSFV-Ind-TN/Trt-11-005 isolate available in the Department was used in animal challenge experiments.

Adaptation of CSFV isolates: All the three CSF viruses were subjected to 40 continuous serial passages in PK-15 cells. Since CSFV do not produce visible CPE, presence of virus in the cells was confirmed by fluorescent antibody virus neutralization test (FAVN) and Polymerase Chain Reaction (PCR) at 10, 15, 20, 25, 30, 35 and 40th passages.

Virus titration: Virus titration at different passages was performed to detect the presence of CSF virus and also to quantify the virus in the passaged cells. Tenfold serial dilutions of culture supernatant were prepared. Three replicates of 100 µl of each serial dilution (10^{-1} to 10^{-10}) were incubated for 4 days on a monolayer of PK-15 cells in a 96 well plate. On the 4th day of virus inoculation, the spent medium was discarded and the monolayers were washed gently in phosphate buffered saline (PBS). The monolayers were fixed in 4% ice cold acetone in PBS for 10 min. After fixing, the plate was washed in PBS and the monolayers were incubated for 1 h with 1:50 dilution of polyclonal reference serum (EU Reference Laboratory, Germany) diluted in PBS Tween. Finally the monolayers were stained with anti-pig FITC (Sigma) and observed under fluorescent microscope. Virus titres were calculated as TCID₅₀ using Reed and Muench method (1938).

Selection of candidate CSF virus vaccine strain: Animal study was performed for the selection of candidate vaccine virus among three PK-15 cell adapted CSFVs as per the standards of OIE Manual (2010). Immunogenicity trial was conducted using two field isolates of CSFV along with one lapinized CSF vaccine strain adapted in PK15 cell line. Four groups, each comprising of three animals (3 months old, Male Large white Yorkshire pigs) were used in the study. Three groups namely, Group 1 (CSFV-TS1), Group

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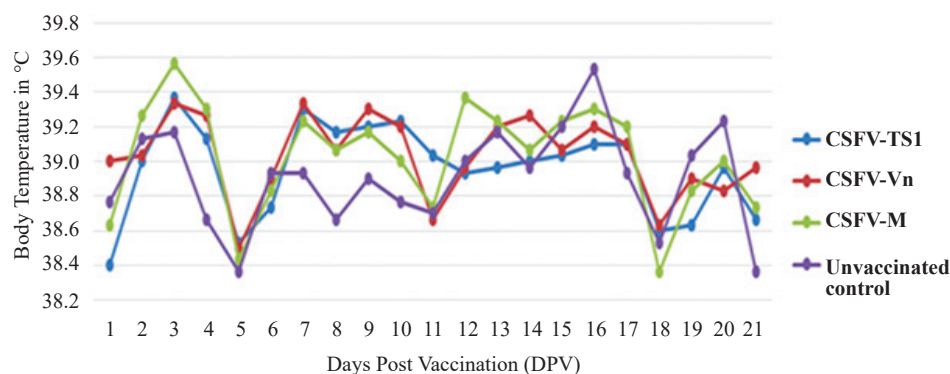


Fig. 1. Body temperature of pigs following immunization with the three CSF viruses showing no abnormal elevation of body temperatures.

2 (CSFV-Vn) and Group 3 (CSFV-M) were administered intramuscularly with the PK-15 adapted CSFV (10^8 TCID₅₀ /1 ml/pig). The fourth group was used as control animals and administered with 1 ml of sterile PBS. Blood samples were collected at weekly intervals from all the animals for assessing blood parameters. All the animals were observed for 21 days for any post-immunization reactions. Nasal swabs were collected from all the animals on 0, 3, 7, 14 and 21 days post immunization (DPI) and screened for virus excretion by RT-PCR using CSFV specific E2 and NS5B gene primers (Rathnapraba *et al.* 2012, Rathnapraba *et al.* 2014). Body weight of all the animals was measured to assess any change in the growth performance due to immunization. All the animals were challenged with a virulent CSF virus (CSFV-Ind-TN/Trt-11-005) 21 days after the immunization and observed for 90 days post-challenge.

Serum samples from all the three groups of animals were collected on 0 (Pre-immunization) and 21 DPI, 14 days post challenge (DPC) and 90 DPC and tested for seroconversion using Prionics CSFV Ab ELISA Kit as per the manufacturer's protocol. Serum samples with a per cent inhibition (PI) value of above 50% were termed as positive and confirmed the presence of CSFV specific antibodies against E2 protein.

Infectivity titres: After 10 passages, the log₁₀ TCID₅₀ titres were found to be 3.5, 4.0 and 4.0 for CSFV-TS1, CSFV-Vn and CSFV-M, respectively. The log₁₀ TCID₅₀ stood at 9.0, 9.5 and 9.8 for CSFV-TS1, CSFV-Vn and CSFV-M, respectively after 40 passages. The results indicated that the viruses adapted well to the PK-15 cells with reasonably good titres after 40 serial passages.

Post-vaccination responses: Two groups of pigs immunized with CSFV-TS1 and CSFV-M virus had shown clinical signs such as reddening of skin at the base of the ear, neck and the ventral part of abdomen during the post immunization period but not in the pigs vaccinated with CSFV-Vn. No abnormal elevation of temperature was recorded in the immunized animals and the temperatures were below 40°C (Fig. 1). After immunization, a mild reduction in WBC count (Fig. 2) was noticed in all the animals but not to

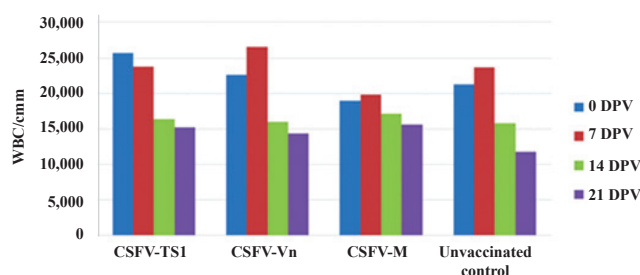


Fig. 2. WBC counts of pigs following immunization with the three CSF viruses indicating no significant changes.

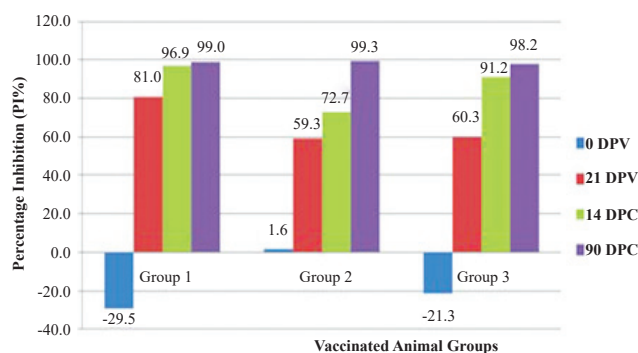


Fig. 3. Seroconversion results of the vaccine candidates (Group 1: CSFV-TS1, Group 2: CSFV-Vn, Group 3: CSFV-M) indicating better sero conversion in Group 2 CSFV-Vn.

the extent of causing leucopenia which is characteristic in CSFV infections (Rout and Saikumar 2012). At 21 days PI, an increase in body weight was noticed in all the animals ranging from 14 to 16 kg indicating that the immunization had no adverse effect on the growth performance of the pigs. As far as virus excretion is concerned, no virus excretion was found in CSFV-TS1 and CSFV-M groups during the observation period of 21 days. However, CSFV-Vn was found in samples collected from two of the three animals in Group 2, on 14 and 21 DPI.

Immunization studies indicated that, among the three CSFV vaccine candidates studied, CSFV-Vn gave higher seroconversion (Fig. 3) than the other two candidates. Elevation of body temperature was observed after challenge in two groups of pigs immunized with CSFV-TS1 and CSFV-M virus but no such raise in body temperature was

observed in pigs immunized with CSFV-Vn virus. Hence, the CSFV-Vn isolate (CSFV-Ind-TN/Veng-09-020) was selected as candidate virus for vaccine production based on animal studies for further experimental studies. More studies with this local candidate strain could help in evolving a better vaccine for the control of Classical swine fever.

SUMMARY

Classical swine fever is an economically important and endemic disease of swine in India. Though vaccination is the best way of controlling the disease, currently available vaccines are not very effective. Cell culture adapted local strains of viral pathogens could be a better choice for large scale vaccine production and better immunity. Two local isolates and one lapinized strain of Classical swine fever virus were adapted to PK-15 cells and assessed for their suitability as a vaccine candidate. The CSFV isolate, CSFV-Ind-TN/Veng-09-020-Vn, with a TCID₅₀ titre of 9.5 after 40 continuous passages was found to give better seroconversion, good protection against virulent CSFV challenge and could be a better candidate vaccine strain.

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