



Survival and inactivation of classical swine fever virus isolated from pigs of Asom, India

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Received: 7 November 2008; Accepted: 23 December 2010

ABSTRACT

The present study was undertaken to evaluate stability and inactivation of the local isolates of classical swine fever virus (CSFV) along with the standard lapinized cell culture adapted CSF virus vaccine exposing to various physical and chemical agents. Vaccine strain and local isolates of CSFV adapted in PK-15 cell line were exposed to different temperatures (4, 27, 60°C), pH (2, 7, 14) and chemicals (2% NaOH, 20% chloroform, 0.5% trypsin) at various time periods. TCID₅₀ of the virus residue was estimated at the end of each treatment. Results showed that all the CSFV isolates lost their virus titre completely within 10 min at 60°C, at pH 2 and pH 14 within 30 min. Viruses exposed to 20% chloroform, 0.5% trypsin and 2% sodium hydroxide, inactivated completely within 60 min. On the other hand, at 27°C, within 60–120 min virus titre was reduced by 0.5–1.0 log₁₀ and at 4°C, titre of the isolates were reduced by 0.5–1.0 log₁₀ within 15–30 days. Exposure to different pH levels showed that CSFV titre remained stable at pH 7 within 15 to 30 min at room temperature. The work provides basic information on physico-chemical properties of cell culture adapted Indian isolates of CSF virus and also a comparison with lapinized CSFV.

Key words: Classical swine fever, CSFV, IPT, PK-15 cell line, Pigs, TCID₅₀

Classical swine fever (CSF), a highly contagious and often fatal disease of swine, affecting domestic and wild pig population, is causing substantial economical loss (Edwards *et al.* 2000). As per the reports of the World Animal Health (2004), 59 new outbreaks of CSF were recorded in India during 2004, and most of the outbreaks were from the North Eastern (NE) states of the country (Sarma *et al.* 2008). In majority of epidemics indirect transmission occurred when susceptible pigs came into contact with mechanical vector like clothings and footwears (Ribbens *et al.* 2007) contaminated with excretions or secretions (De Smit *et al.* 1999). Viability of the virus depends on initial amount of virus, temperature, pH, humidity, exposure of various chemicals (Edwards 2000) and most likely other factors like properties of the virus strain (Depner *et al.* 1992). Studies on viability of CSFV are concentrated in meat so far (Edwards 2000). Survivability of the virus in environment, *in vitro* and susceptibility to various chemicals and disinfectants need extensive study. There is no information about susceptibility and inactivation of Indian isolates of classical swine fever virus to various physico-chemical factors. In this report survival and inactivation of local isolates of CSFV along

with the standard lapinized cell culture adapted CSFV vaccine strain exposed to various temperatures, pH and chemicals were studied.

MATERIALS AND METHODS

Isolation of CSFV: Lapinized cell culture adapted-CSFV vaccine and field samples collected from natural outbreaks of CSF were processed for isolation of CSF virus in PK-15 cell line (National Centre for Cell Science, Pune). The cells were grown in minimum essential medium supplemented with 10% fetal bovine serum, 100 IU of penicillin G / ml, and 100µg of streptomycin / ml. Cells were routinely subcultured with 0.25% trypsin (1: 250). Presence of CSFV in cell culture fluid was confirmed by direct fluorescent antibody technique (FAT), indirect immunoperoxidase linked assay (IPT) as per European Union Diagnostic Manual (Anonymous 2002) and sandwich-ELISA as per Sarma and Sarma (1996). Isolated virus was given subsequent serial passages to adapt in cell line and to increase the titre.

Titration of virus: Virus titre was determined by indirect immunoperoxidase linked assay (IPT; Anonymous 2002). Briefly, cell monolayer cultured in 96 wells micro-titre plate was infected with CSFV. For each dilution of virus, 4 wells were taken and virus was diluted serially. After 72 h of incubation, the cells were fixed at 70–80°C for 2–3 h. Pig

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anti-CSFV antibody (1: 800) diluted in PBS-T was added in 50 μ l volumes and followed by HRPO conjugate (1: 1000) to each well. A solution of 3-amino-9 ethyl carbazole (AEC) containing 3% H₂O₂ was used as the substrate. The reaction was stopped after 15–30 min at room temperature by washing the monolayers with 1/3 PBS: H₂O. Infected cells stained dark brown, and results of this assay was examined under low power microscope. The CSFV titre (TCID₅₀/ml) was calculated from the number of positive wells observed at each dilution using the Spearman-Kärber method (Finney 1978). Virus infectivity titre was expressed as tissue culture infective dose (TCID₅₀) per volume of virus suspension.

Physico-chemical properties of virus: Five CSF virus isolates recovered from spleen (Lab no. J4/192, J4/193, DMJ4/234) and kidney (DMJ4/234, SLP4/230) showing high titre ($\geq 3.00 \log_{10}$ TCID₅₀/ ml of cell culture fluid) along with the standard lapinized CSF virus adapted in cell culture were exposed to different temperatures (4, 27, 60°C), pH (2, 7, 14) and chemicals (20% chloroform, 0.5% trypsin and 2% sodium hydroxide) for different time periods. Virus titre of each isolate was determined before and after each treatment.

Susceptibility to temperature: Survivability and inactivation of CSFV exposing to different temperatures was studied as per Liess (1981). For each treatment 3 ampules containing 1 ml virus suspension were taken. One set of virus suspensions were kept at 60°C in a water bath for 0, 10 and 30 min, another set at 27°C for 0, 60, 120 min and remaining set were kept at 4°C for 0, 1, 15 and 30 days.

Susceptibility to pH: Studies on the effect of the acid and alkali upon CSFV were determined as per Liess (1981). The pH of the virus suspensions were adjusted to 2, 14 and neutral (7.0) by adding either 0.1N HCl or 0.1N NaOH. For each treatment 3 ampules were used and ampules containing the virus suspensions were exposed at 4°C for 0, 15 and 30 min.

Susceptibility to chemicals: Susceptibility of CSFV to 20% chloroform was determined as per Horzinek *et al.* (1976) and effect of 2% sodium hydroxide was determined as per Liess (1981). The virus treated with 0.2 ml of 20%

chloroform/ml and 0.02 ml of 2% sodium hydroxide/ml respectively were exposed for 0, 15, 30 and 60 min at 27°C. Effect of 0.5% trypsin on CSFV was evaluated as per Dinter (1963). The virus containing ampules were exposed to 0.1 ml of 0.5% trypsin / ml of virus suspension and allowed to react for 0, 15, 30 and 60 min at 37°C.

At the end of each treatment, viability of the virus isolates was evaluated by giving passage in PK-15 cell line and confirmed by immunoperoxidase linked assay (IPT).

RESULTS AND DISCUSSION

Physico-chemical characterization: Exposure to 4°C indicated that the virus titres remain unchanged within 24 h and lost its titre ranging from 0.5 to 1.0 $\log_{10}$ within 15 to 30 days (Table 1). This finding confirms that Indian isolates of CSFV are reasonably stable at low temperature. Thus it facilitates handling of virus at low temperature in the laboratory and shipment of samples for diagnosis. In general, diagnostic samples should be kept cool (4°C wherever possible). Horzinek (1981) observed that at 4°C reduction of 1.6 $\log_{10}$ titre occurs after 4 days. Harkness (1985) reported no loss of titre in cell culture fluid even after 180 days at 4°C. Similarly the thermostability of the cell culture adapted virus at room temperature (27°C) showed that at room temperature CSF virus titre drop 0.5–1.0 $\log_{10}$ within 60 to 120 min (Table 1). Therefore it is not too deleterious to handle the virus at room temperature for a short period. Horzinek (1981) reported that at room temperature, a 2.7 $\log_{10}$ drop of titre of CSFV isolates were observed within 3 days. Present study further showed that Indian isolates of CSFV inactivated completely at 60°C within 10 min. Turner *et al.* (2000) also reported inactivation of CSFV at 60°C. Spreading of the virus along with the infected meat and feeding swill is an important mode of infection in CSFV epidemic (Terpstra 1988). Adequate heat treatment of meat and swill can reduce spread of virus infection. Swine fever virus is readily inactivated by pasteurization and cooking (Terpstra and Krol 1976). In pork processing unit, smoking temperature is maintained at 60°C. Our results support that there is less chance of spread of CSF virus through smoked pork and pork products.

Table 1. Thermostability of CSFV to various temperature exposures for different time periods

Sample no.	Virus titre (TCID ₅₀ /ml) after exposing to temperature at									
	4°C				27°C			60°C		
	0 min	Day 1	Day 15	Day 30	0 min	60 min	120 min	0 min	10 min	30 min
J4/192	5.25	5.25	4.50	3.50	5.25	4.75	4.25	5.25	BDL	BDL
J4/193	4.75	4.75	3.75	3.25	4.75	4.25	3.75	4.75	BDL	BDL
DMJ4/234	4.50	4.50	3.50	3.00	4.50	4.00	3.50	4.50	BDL	BDL
SLP4/230	3.50	3.50	3.00	2.50	3.50	3.00	2.50	3.50	BDL	BDL
DMJ4/234	4.50	4.50	3.75	3.25	4.50	4.00	3.50	4.50	BDL	BDL
Vaccine strain	5.50	5.50	5.00	4.50	5.50	5.00	4.50	5.50	BDL	BDL

BDL, Below detectable level.

Table 2. Stability of CSFV at various pH exposed for different time periods

Sample no.	Virus titre (TCID ₅₀ /ml) after exposing to pH at 27°C								
	2.0			7.0			14.0		
	0 min	15 min	30 min	0 min	15 min	30 min	0 min	15 min	30 min
J4/192	5.25	2.00	BDL	5.25	5.25	5.25	5.25	1.75	BDL
J4/193	4.75	1.50	BDL	4.75	4.75	4.75	4.75	1.25	BDL
DMJ4/234	4.50	1.25	BDL	4.50	4.50	4.50	4.50	BDL	BDL
SLP4/230	3.50	BDL	BDL	3.50	3.50	3.50	3.50	BDL	BDL
DMJ4/234	4.50	1.25	BDL	4.50	4.50	4.50	4.50	BDL	BDL
Vaccine strain	5.50	2.50	BDL	5.50	5.50	5.25	5.50	1.50	BDL

BDL, Below detectable level.

Table 3. Effect of 2% sodium hydroxide, 20% chloroform and 0.5% trypsin on CSFV incubated for different time periods

Sample no.	Virus titre (TCID ₅₀ /ml) after exposing to chemicals and enzyme											
	NaOH (2%) at 27°C				Chloroform (20%) at 27°C				Trypsin (0.5%) at 37°C			
	0 min	15 min	30 min	60 min	0 min	15 min	30 min	60 min	0 min	15 min	30 min	60 min
J4/192	5.25	3.50	BDL	BDL	5.25	3.75	1.25	BDL	5.25	4.25	2.00	BDL
J4/193	4.75	2.00	BDL	BDL	4.75	3.25	BDL	BDL	4.75	3.75	1.25	BDL
DMJ4/234	4.50	1.75	BDL	BDL	4.50	3.00	BDL	BDL	4.50	3.50	BDL	BDL
SLP4/230	3.50	BDL	BDL	BDL	3.50	2.50	BDL	BDL	3.50	2.50	BDL	BDL
DMJ4/234	4.50	1.75	BDL	BDL	4.50	2.75	BDL	BDL	4.50	3.50	BDL	BDL
Vaccine strain	5.50	2.50	BDL	BDL	5.50	2.25	BDL	BDL	5.50	3.50	1.50	BDL

BDL, Below detectable level.

There was no loss of titre at pH 7 within 15 to 30 min (Table 2). On the one hand, virus titres were lost completely at pH 2, within 15–30 min and on the other hand virus titres were lost completely between pH 7 and 14 within 15–30 min. The present study confirms that Indian isolates of CSFV including vaccine strain are stable at neutral pH but inactivated at extreme acid and alkaline pH. Edwards (2000) also observed that CSFV is stable at neutral to slightly alkaline pH and rapidly inactivated at pH 3 or below and above pH 10.

As an enveloped virus, swine fever virus is inactivated by organic solvents, such as ether or chloroform (Moennig 1988). Selection of effective organic solvents as decontaminants of the swine fever virus bears practical implication in field. As always with disinfectants, an essential first step should be cleaning of the area, and in swine fever this will probably achieve most of the desired decontamination. In this study 2% sodium hydroxide, the most commonly used disinfectant inactivated CSFV at 27°C (Table 3) within 30 min. Similarly CSF virus when exposed to 20% chloroform at 27°C (Table 3) gradually reduced the titre within 15 min and complete loss of titre was observed within 30 min except in vaccine strain. Yang *et al.* (2004) reported loss of titre of Japanese Encephalitis virus belonging to family *Flaviviridae* with 20% chloroform within 1 h at 22°C. Studies on the effect of 0.5% trypsin at 37°C reveals

(Table 3) gradual drop of titre within 15 min and inactivated completely within 30–60 min. Dinter (1963) reported loss of titre of CSFV while treating the virus with 0.5% trypsin at 37°C within 60 min. Laude (1977) reported marked reduction of titre of CSFV in presence of trypsin.

The present investigation provides basic information on physico-chemical properties of cell culture adapted CSF virus. Comparing to the existing lapinized vaccine strain of CSFV and other European isolates, Indian isolates of CSFV possess similar properties on stability and inactivation of virus exposing to heat, pH and chemicals. However, further study on physico-chemical characteristics of field isolates will provide information about selection of right physical and chemical inactivants against CSF virus.

ACKNOWLEDGEMENTS

The authors are thankful to the Department of Biotechnology, Govt of India for providing financial help under the project entitled “Molecular and immunological characterization and phylogenetic analysis of Indian isolates of classical swine fever virus” to carryout the research.

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