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Pathogenicity of porcine sapelovirus infection in mice

SWATI KUMARI[1], P K RAY[2], RAHUL SINGH[3] and G SAIKUMAR[4]

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

Porcine Sapelovirus (PSV) is a RNA virus belonging to a new genus *Sapelovirus* of family *Picornaviridae.* PSV has been isolated from India in 2016. In the present study, mice experiment was conducted to detect the ability of PSV to infect mice and its ability to induce pathological lesions. The intestinal and extra intestinal spread of the PSV virus in three week-old Swiss albino mice inoculated with PSV virus quantified by probe based real time PCR are described. Herein, three groups were made with 10 mice per group (both sex). The first group was infected through oral route (8×10^6, $TCID_{50,}$ 240 µl/mice) while the second through intra-peritoneal route (8×10^6, $TCID_{50,}$ 240 µl/mice) and the third group was inoculated with PBS of neutral pH orally and intra-peritoneal route. Seven mice (each from oral and intraperitoneal route and three from control group were sacrificed at 5th, 7th, 9th, 12th, 15th, 17th, 21st day post infection (DPI). Indian strain was able to replicate in mice organs up to 15 DPI in oral route and 9 DPI in intraperitoneal route. By real-time reverse transcription (RT) PCR, PSV was detected in most of the organs but with highest viral load in the small intestine and large intestine than extra-intestinal organs in the orally infected mice. In addition, this Indian strain is enteropathogenic but could spread to the bloodstream from the gut and disseminate to extra-intestinal organs. These results will contribute to our understanding of PSV pathogenesis.

Key words: Histopathology, Mice, Pathogenesis, PSV, qPCR, RT PCR

Sapelovirus A (SV-A), formerly known as porcine sapelovirus (PSV), member of a new genus *Sapelovirus*, belonging to family *Picornaviridae* is known to cause diarrhoea, respiratory signs, encephalitis and skin lesions (Adam *et al.* 2015). PSV is non-enveloped, spherical, about 30 nm in diameter, has linear genome, non-segmented, single-stranded positive-sense RNA with a length of 7.5–8.3 kb nucleotides (Lan *et al.* 2011, Schock *et al.* 2014). PSV had been commonly isolated from faeces of healthy piglets (Buitrago *et al.* 2010, Chen *et al.* 2012, Kim *et al.* 2016, Ray *et al.* 2018, Bai *et al.* 2018). Routine laboratory diagnosis of PSV infection depends mainly on virus isolation and characterization, but this technique is less effective and time-consuming. Several molecular methods for detecting PSV have been developed such as reverse transcription PCR (Palmquist *et al.* 2002), nested reverse transcription PCR (Zell *et al.* 1997), and real-time quantitative PCR (Krumbholz *et al.* 2003). However, only few experimental studies of PSV had been reported (Lan *et al.* 2011, Kim *et al.* 2016)

In India, the virus has been isolated /detected from faeces of healthy pigs as well as those affected with diarrhoea, respiratory signs, encephalitis, skin lesions and fertility disorders (Ray *et al.* 2018). The present study was carried out to evaluate the pathogenesis and pathology of PSV infection in Swiss albino mice as the pathogenicity and/or host range limitation of PSV has been poorly characterized.

Present address: [1]MVSc Scholar (swati.chaudhary.vet@gmail.com), [2,3]PhD Scholar (rayprdeep56@yahoo.co.in, drrahul.sigh1987@gmail.com), [4] Principal Scientist Incharge PME Cell (saikumarvri@gmail.com), Molecular Pathology Laboratory, Swine Disease Laboratory, Division of Pathology.

MATERIALS AND METHODS

Ethical statement: All the experimental procedures on animals were carried out according to the recommendations and approval of the Institutional Animal Ethics Committee (IAEC) as per the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

Virus: Sapelovirus/IVRI/SPF-c-6/2015 strain (Strain C-6), accession number (KY053835) of an Indian isolate of PSV was obtained from Swine Disease Laboratory, Division of Veterinary Pathology, IVRI, Izatnagar.

Experimental infection in mice: The experimental infection was conducted in healthy three week-old mice (30) which were tested negative for PSV infection before conducting the experiment, were obtained from lab animal resource (LAR), IVRI. The animal experiment was carried out by infecting mice with the tissue culture infective dose ($TCID_{50}$) of PSV through oral and intra-peritoneal route. Three groups were made each having 10 mice (both sex). The first group was infected through oral route (8×10^6, 240

µl/mice) directly into mouth, second through intra-peritoneal route (8×10^6, 240 µl/mice), and third (control group) was inoculated with PBS through intra-peritoneal route. Duration of experiment was 21 days. Seven mice (each from oral and intraperitoneal route and three from control group were humanely sacrificed at 5th, 7th, 9th, 12th, 15th,17th, 21st day post infection (DPI). Infected mice were observed for any clinical signs by gentle abdominal palpation every day. Observations were recorded and documented daily. Euthanasia was performed by sodium pentobarbital overdose followed by cervical dislocation. The systematic dissection of mice was carried out for any gross lesion in organs with the tissue collection of the intestines, liver, kidneys, spleen, lungs and brain in 10% neutral buffered formalin (NBF) for histopathology and without any preservative for molecular pathology at -80°C. RT PCR and qRT PCR were carried out to confirm the presence of virus in different organs.

RNA extraction: Total RNA was extracted from tissues using commercial TRIzol® Reagent (Thermo Fisher Scientific, USA) method essentially following manufacturer's protocol. All the RNA samples extracted were quantified by NanoVue plus (Thermo Fisher Scientific, USA) and stored at -80°C until further use. The cDNA synthesis was performed as described earlier in our study (Ray *et al.* 2017) and the synthesized cDNA was stored at -20°C till further use for RT-PCR and qPCR with the use of laboratory designed primers (Table 1).

PCR and real time PCR amplification: PCR reaction was carried out in 0.2 ml PCR tubes containing reaction mixture of 6.0 µl of PCR Master Mix 2× (Takara, City), 0.5 µl each of forward (SapF) primer and reverse primer (SapR) (10 pmol/µl) , 1 µl of cDNA (200 ng/µl) and 12.00 µl of nuclease-free water. The contents were mixed thoroughly and spun briefly. The tubes were then placed in a thermocycler and PCR cycling conditions were standardized with initial denaturation of 95°C for 5 min, followed by 35 cycles of 94°C for 10 sec, 53°C for 20 sec at annealing, 72°C for 30 sec, and a single cycle of final extension at 68°C for 7 min. Amplified products were resolved by agarose gel electrophoresis (1% w/v) at 100 V for 1 h in TAE buffer with 0.5 µg/ml ethidium bromide and viewed under UV transilluminator (Geldoc, USA). TaqMan PCR assay was performed using (Kappa probe fast) Universal qPCR Kit (KAPA Biosystems, USA) in Agilent Aria MX Real-Time PCR System as per manufacturer's recommendation. Reaction mixture (25 µl) consisted of KKapa qPCR Probe Fast (10 µl), PSV F1 (10 µM) (0.5 µl), PSV R1 (10 µM) (0.5 µl), PSV P1 (10 µM; Probe) (1.0 µl), template cDNA (3.0 µl) and nuclease-free water (10.0 µl) with the thermal profile of initial denaturation at 95°C for 3 min followed by denaturation temperature of 95°C for 30 sec and annealing at 55°C for 20 sec.

Ethical statement: All the experimental procedures on animals were carried out according to the recommendations and approval of the Institutional Animal Ethics Committee (IAEC) as per the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

RESULTS AND DISCUSSION

There is no report of experimental PSV infection in mice. In the present study, mice experiment was done to assess the pathogencity of virus. All the infected group mice showed no clinical sign and no mortality during the experiment. Animals were sacrificed at 5th, 7th, 9th, 12th, 15th, 17th and 21st DPI. Systematic necropsy was carried out but no gross lesion was detected. In histopathological changes, interstitial pneumonia with thickening of inter-alveolar septa due to moderate infiltration of mononuclear cells was observed in lungs (Fig. 1b, c). In the brain, perivascular infiltration and gliosis were seen in mice of both groups (Fig. 1e, f) except control group. In IP route of infection, mice showed multifocal random aggregation of mononuclear cell infiltration in the liver parenchyma (Fig. 1h,i). In the intestine, villous atrophy, crypt hyperplasia, necrosis and sloughing of villi along with mononuclear inflammatory cell infiltration in the lamina propria was observed on histopathological examination in both groups (Fig. 2) except control group.

In the present study, the most significant lesion was found in the intestines of mice. Kim *et al.* (2016) demonstrated Korean SV-A strain in experimental infected piglets and chicks. The lesions were non-suppurative myelitis, encephalitis, and pneumonia in piglets, but not in chicks. Previous studies have indicated that PSV can cause pneumoenteritis, polioencephalomyelitis and reproductive disorders in pigs (Lan *et al.* 2011, Schock *et al*. 2014). All the samples collected for the study of experimental molecular pathology were screened by RT PCR (Fig. 3) and qRT-PCR. Virus was detected in significant concentration from the collected organs showing PSV replication in all these organs of mice. But virus could not

Table 1. Sequences of oligonucleotide primers and probes

Primer name	Sequence	Product size	Region	References
SapF	5'- CTTTGGTGATTCGGCGACTGG-3'	215 bp	5' UTR	Lab designed
SapR	5'-ACTGACTATACTAGTTACAGGCG-3'			
PSV Probe	5'-/56-FAM/CCAGCCGCGACCCTATCAGG/ 36-TAMSp/-3'	143 bp	5'UTR	Lab designed (unpublished)
	5'-GGAAACCTGGACTGGGYCT-3'			
	5'-ACACGGGCTCTCTGTTTCTT-3'			

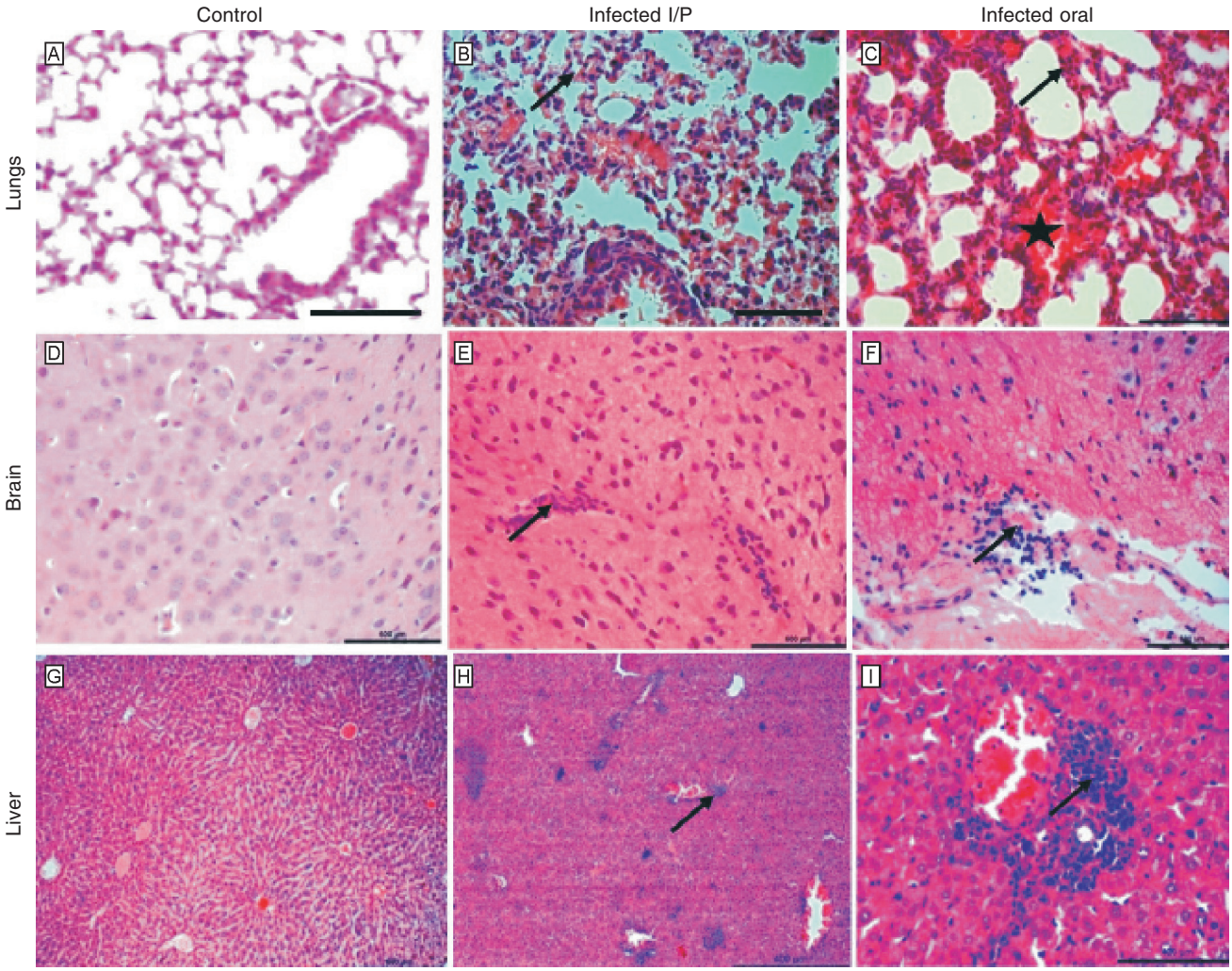

Fig. 1. Histopathological changes in different organs of mice after inoculated I/P and oral route with PSV. (**A**) Control group mice, (**B**) Interstitial pneumonia comprises mononuclear infiltration with moderate thickening of interalveolar septum (H&E, 400×), (**C**) Interstitial pneumonia with inflammatory mononuclear cell infiltration in the interalveolar septa and engorgement of pulmonary vessels. (H&E, 400×), (**D**) Control mice, (**E**) Perivascular infiltration (H&E, 400×), (**F**) Mononuclear infiltration around the blood vessels and gliosis (H&E, 400×), (**G**) Control group, (**H**) Multifocal random aggregation of mononuclear inflammatory cells (H&E, 100×), (**I**) Aggregation of mononuclear cells around central vein (H&E, 400×).

Table 2. RT- PCR and q RT-PCR on tissue specimens obtained from mice which were infected through peritoneal route

Organ	PCR and qRT-PCR on mice infected with PSV through IP route							
	5^{th} PDI RT-PCR	5^{th} PDI qPCR (no. of copy/µl)	7^{th} PDI RT-PCR	7^{th} PDI qPCR (no. of copy/µl)	9^{th} PDI RT-PCR	9^{th} PDI qPCR, (no. of copy/µl)	15^{th} PDI RT-PCR	15^{th}PDI qPCR (no. of copy/µl)
Brain	Positive	1×10^2	Positive	2×10^2	Positive	2.5×10^2	Negative	–
Heart	Positive	9×10^2	Positive	9×10^2	Positive	7.5×10^2	Negative	–
Lung	Positive	2×10^3	Positive	2×10^3	Positive	7×10^2	Negative	–
Liver	Positive	1×10^2	Positive	1×10^2	Positive	9×10	Negative	–
Spleen	Positive	4×10^3	Positive	4×10^3	Positive	4×10^3	Negative	–
Kidney	Positive	4×10^3	Positive	4×10^3	Positive	4×10^3	Negative	–
Small intestine	Positive	2×10^6	Positive	1×10^6	Positive	9×10^5	Negative	–
Large intestine	Positive	2.5×10^4	Positive	9×10^4	Positive	9×10^4	Negative	–

be detected after 12 dpi in intra peritoneal route (Table 2) and after 15 dpi in oral route (Table 3). qRT-PCR results indicated that virus multiplication is higher in the small intestine than in large intestine in IP route of infection. Viral load was high in small intestine but in subsequent days it decreased with increase in large intestine and lungs in oral route of infection. These methods detected PSV in organs of mice such as brain, heart, lung, spleen, kidney and liver

Table 3. RT-PCR and q RT-PCR on tissue specimens obtained from mice which were infected through oral route

Organ	PCR and qRT-PCR on mice infected with PSV through oral route									
	5th PDI RT-PCR	5th PDI qPCR (no. of copy/µl)	7th PDI RT-PCR	7th PDI qPCR (no. of copy/µl)	9th DPI RT-PCR	9th DPI qPCR (no. of copy/µl)	15th DPI RT-PCR	15th DPI qPCR (no. of copy/µl)	17th DPI qPCR (no. of copy/µl)	21st DPI qPCR (no. of copy/µl)
Brain	Negative	–	Positive	1×10^2	Positive	4×10^2	Positive	4×10^2	–	–
Heart	Negative	–	Negative	9×10	Negative	9×10	Negative	2.5×10	–	–
Lung	Negative	–	Negative	–	Negative	–	Positive	2×10^2	–	–
Liver	Positive	5×10^3	Positive	9×10^3	Positive	9.5×10^2	Positive	9.5×10	–	–
Spleen	Positive	9×10^2	Positive	2×10^2	Positive	2.75×10^2	Positive	1×10^2	–	–
Kidney	Positive	7.5×10^3	Positive	9×10^2	Positive	4×10^2	Negative	9×10	–	–
Small intestine	Positive	9×10^3	Positive	3×10^3	Positive	4.75×10^3	Positive	2×10^2	–	–
Large intestine	Positive	4×10^2	Positive	4×10^3	Positive	4×10^2	Positive	2.5×10^5	–	–

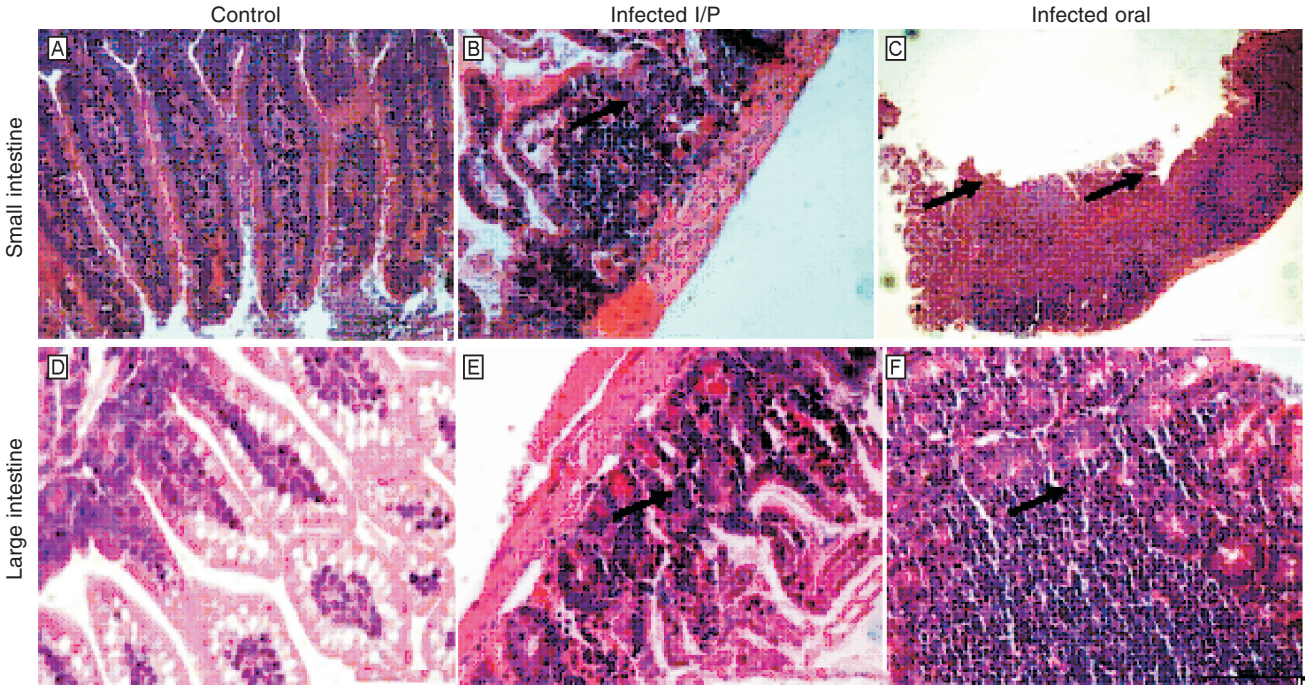

Fig. 2. Histopathological changes in small and large intestine of mice after inoculated i/p and oral route with PSV. (**A**) Control mice, (**B**) Mononuclear cell infiltration in the lamina propria (H&E, 400×), (**C**) Mononuclear cell infiltration in the lamina propria and crypt hyperplasia with sloughing of villi (H&E, 400×), (**D**) Control mice, (**E**) Sloughing of villi and mononuclear cell infiltration in the lamina propria of intestine (H&E, 400×), (**F**) Mononuclear cell infiltration in lamina propria with epithelial cell hyperplasia (H&E, 400×).

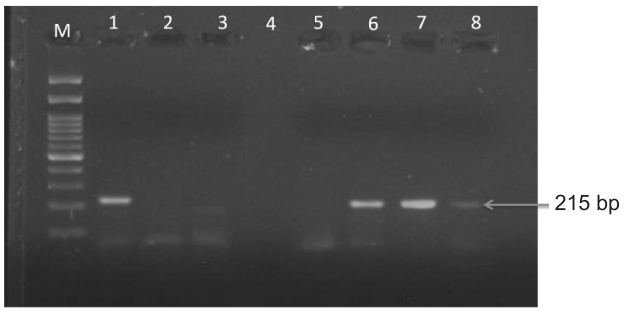

Fig. 3. RT-PCR screening of PSV experimentally infected mice sample targeting 5' UTR specific region. Lane 1 (Postive control), lanes 2,3,4, negative sample; lane 5, negative control; lanes 6, 7, 8, lanes positive sample and lane M, 100 bp ladder.

and intestine. PSV was found to persist in mice tissues upto 9th DPI in mice infected intraperitonealy (Table 2) and up to 15th DPI through oral route (Table 3).

Further studies are required to know the pathogenicity of this virus and also to examine the predilections of age and inoculation routes, as well as antigenic and genomic differences. As in other picornaviruses, BALB/c adult mice are highly susceptible to Foot and Mouth Disease virus (FMDV) infection when the virus was administered subcutaneously or intraperitoneally. This mouse model was used to study FMDV pathogenesis (Salguero *et al.* 2005). An experimental animal model of Encephalomyocarditis virus (EMCV) in eight-weeks-old male BALB/c had been reported. EMCV could replicate in brain tissues and induce acute encephalitis in BALB/ c mice (Yuan *et al.* 2015). The

results of experimental infection with DPV (Avian Sapelovirus) by various routes indicated that DPV did not cause mortality in day-old ducklings but has potential to cause growth inhibition (Tseng and Tsai 2007). In conclusion, this Indian strain PSV is enteropathogenic but could spread to the bloodstream from the gut and disseminate to extra-intestinal organs. These results could be contribute to our understanding of PSV pathogenesis.

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