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Genetic Characterization and Pathology of Porcine Teschovirus (PTV) in India

Shailesh Kumar Patel[1,2], Jigyasa Rana[3], Mamta Pathak[2], Alok Singh[2], Aditya Agrawal[4], G. Saikumar[2]

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ABSTRACT

Background: The porcine teschovirus (PTV) is common swine pathogen which causes a wide range of illnesses ranging from asymptomatic infection to acute fatal encephalomyelitis, diarrhoea and pneumonia. Despite of its economical importance very limited studies are available on the pathology of PTV. The present study was conducted with the objective to investigate the PTV infection and associated pathology in piglets.

Methods: A total of 78 piglets below 3 months of age were necropsied and representative tissue samples along with intestinal content were collected for histopathological examination and molecular investigation during April 2019 to November 2020. The 5′ UTR region of PTV was amplified via RT-PCR and further confirmed by sequencing. Thereafter, genetic characterization of the Indian PTV isolate was done on the basis of 5′ UTR gene.

Result: A total of seven out of 78 cases were found positive for the PTV. On necropsy the intestinal wall was thinned and distended with yellowish coloured diarrhoeic content. Congestion of serosal and mucosal vessels along with severe meningeal congestion was observed. Microscopic lesions included congestion of mucosal vessels and destruction of villous structure of jejunum along with perivascular cuffing, focal gliosis, neuronophagia, congestion of meningeal and cerebral vessels and interstitial pneumonia. The phylogenetic analysis revealed that the isolate PTV/India/IVRI-381/2020 (MW022462) and PTV/India/IVRI-1093/2020 (MW022463) were clustered with PTV-13 strain wild boar/WB2C-TV/2011/HUN (JQ429405) and PTV-2 strain Vir 6711-12/83 (AF296107) of Hungary and Germany respectively. This study reported the genotypic and pathological investigation of PTV from Indian piglets. Further explorative surveillance along with studies in suitable cell lines and animal model will pave the way for better understanding of PTV among Indian pigs.

Key words: Pathology, Pigs, Porcine teschovirus.

INTRODUCTION

As per the 20th livestock census of India the estimated population of pigs is 9.06 million which is declined by a significant value of 12.03% over previous census (BAHS, 2019). This decline in pig population is a serious matter of concern as pig farming has great potential to ensure economic and nutritional security for the weaker sections of the society. Additionally, the profitability of pig farming is also reduced drastically due to occurrence of various infectious diseases. The porcine enteric picornaviruses such as porcine teschovirus (PTV), porcine sapelovirus (PSV) and porcine kobuvirus are responsible for severe diarrhoea with reduced performance and high mortality leading to significant economic losses (Patel *et al*., 2023; Patel *et al*., 2023).

The PTV belongs to the family Picornaviridae comprised of small, icosahedral, non-enveloped viruses with a single positive strand RNA genome. The virus circulates worldwide in asymptomatic domestic pigs of all ages (Prodelalova, 2012). The PTV has been reported from domestic pigs and wild boars in Asia, Europe, North America, Central America and South America (Donin *et al*., 2014). Recently, for the first time serotype 5 and serotype 8 of PTV along with a new variant were isolated from the faeces of pigs in India (Ray *et al*., 2020).

[1]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Rewa-486 001, Madhya Pradesh, India.
[2]Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243 122, Uttar Pradesh, India.
[3]Department of Veterinary Anatomy, Faculty of Veterinary and Animal Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur-231 001, Uttar Pradesh, India.
[4]Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Rewa-486 001, Madhya Pradesh, India.

Corresponding Author: Shailesh Kumar Patel, Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Rewa-486 001, Madhya Pradesh, India.
Email: shaileshpatel624@gmail.com

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Porcine teschoviruses are ubiquitous in distribution among pig herds. A wide range of illnesses associated

with these viruses such as mild to severe encephalomyelitis, diarrhoea and respiratory distress in pigs has been reported all over India (Ray *et al*., 2020). Microscopic lesions of the infection include mucosal congestion and villous atrophy in the intestines along with neuronal degeneration, congestion of meningeal and cerebral vessels and interstitial pneumonia. Lesions in the brain stem and spinal cord are characterized by multifocal gliosis, neuronophagia and perivascular cuffs composed of lymphocytes, macrophages, plasma cells and rare eosinophils (Salles *et al*., 2011).

Despite of this, only preliminary work is done on the pathogenesis and pathology of PTV, hence a thorough investigation is crucial to establish the disease pattern of this virus in Indian pigs. Keeping the above facts in the consideration this study was conducted for genetic characterization of PTV along with establishment of its pathology in Indian pigs.

MATERIALS AND METHODS

Collection of samples

This study included the intestinal content and tissue samples from a total of 78 naturally died pigs carcasses presented for the post-mortem examination to the Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly during April 2019 to November 2020. The study included carcasses of either sex of less than three months of age with or without the history of diarrhoea. The carcasses were subjected to detailed necropsy examination and the gross findings were recorded carefully. The representative tissue samples from lung, liver, heart, spleen, kidney, brain, intestines, tonsil and lymph nodes were properly collected as individual samples on ice and 10% neutral buffered formalin for molecular investigation and histopathological examination respectively.

Histopathological examination

Histopathological processing, 0.5-1.0 cm thick formalin fixed tissue pieces were washed overnight under running tap water followed by dehydration through ascending grades of alcohol. After dehydration, clearing was done with acetone and benzene followed by embedding in paraffin wax blocks. Thereafter, 4-5 ìm thick paraffin embedded tissue sections were made by microtome and stained with routine haematoxylin and eosin stain using standard protocol described by Bancroft and Gamble, (2008). The stained tissue sections were thoroughly examined using microscope and the histopathological lesions were carefully recorded and photographed digitally (Olympus BX41, USA).

Molecular examination

Total RNA extraction from tissue samples

The total RNA was extracted from tissue samples using commercial TRIzol® Reagent (Life technologies) which is a modified and improved version of RNA extraction employing guanidium isothiocyanate and phenol as the monophasic solution (Chomcynski and Sacchi, 1987). Approximately 100 mg of tissue samples were homogenized properly in ice with 500 µl of TRIzol® reagent for extraction of total RNA. Thereafter, the homogenized suspension was used for RNA extraction using modified manufacturer's standard protocol described by Patel *et al*. (2023). All the extracted RNA samples were quantified by NanoVue plus (Thermo Fisher Scientific, USA) and the purity of RNA was measured by A260/230 and A260/280 ratio.

First strand cDNA (complementary DNA) synthesis

The extracted RNA samples were subjected to first strand cDNA synthesis by random priming using the genetically modified Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLVRT) (Revert Aid First Strand cDNA Synthesis Kit, Thermo Scientific) as described earlier by Patel *et al*. (2023) and stored at -20°C for further use.

Polymerase chain reaction (PCR) amplification

The amplification of 5' UTR region of PTV was carried out *via* RT-PCR using the primer pair PEV 1a: 5′-AGTTTTGGATTATCTTGTGCCC -3′ and PEV 1b: 5′-CCAGCCGCGACCCTGTCAGGCAGCAC -3′ (Zell *et al*. 2000). All the samples were also screened for porcine sapelovirus (PSV) and enterovirus G (EV-G) using specific primers. PCR reaction (12 µl) was carried out in 0.2 ml PCR tubes containing 6.0 µl of DreamTaq™ Green PCR Master Mix (2X), 0.5 µl of PEV 8 g primer (10 pmol/µl), 0.5 µl of PEV 8h primer (10 pmol/µl), 2.0 µl of cDNA (100 ng/µl) and 3.0 µl of nuclease-free water using a thermocycler (S1000™ Thermal Cycler, BIO-RAD Laboratories India). The PCR was carried out with an initial denaturation of 95°C for 3 minutes; 45 cycles of 95°C for 30 seconds (denaturation), 51°C for 20 seconds (annealing) and 68°C for 40 seconds (extension); and a final extension at 68°C for 7 minutes. Thereafter, the amplified PCR products were visualized by agarose gel (1.5% w/v) electrophoresis after staining with 0.5 ìg/ml ethidium bromide at 90 V for 60 min under UV transilluminator (Geldoc, USA).

Sequencing of RT-PCR amplicons

The amplified PCR products was confirmed by direct sequencing of purified DNA using specific primer used for amplification at DNA sequencing facility of Eurofins, Bangalore. The generated sequencing data was received as the coloured electropherograms and text files which was analysed and processed further for GenBank submissions and phylogenetic analysis.

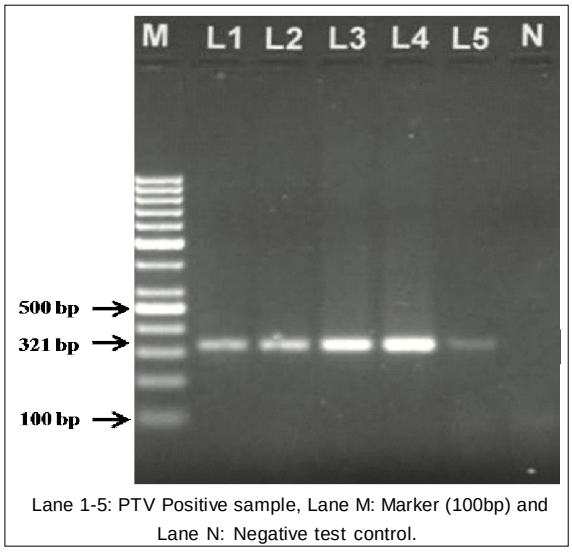
Phylogenetic analysis

The phylogenetic analysis based on 5' UTR was performed to establish the genotypes of the sequenced PTV strains. Thereafter, sequences of various PTV isolates

corresponding to other genotypes from different countries were retrieved from GenBank and used as input sequences along with the sequence of PTV isolates found in the present study for multiple sequence alignment. The sequence alignment was carried out using ClustalW programme of MEGA v.6 software followed by construction of a phylogenetic tree (Tamura *et al*., 2013). The Neighbor-joining (NJ) was applied as the statistical method and the reliability of the constructed tree was determined by bootstrap replicates of 1000.

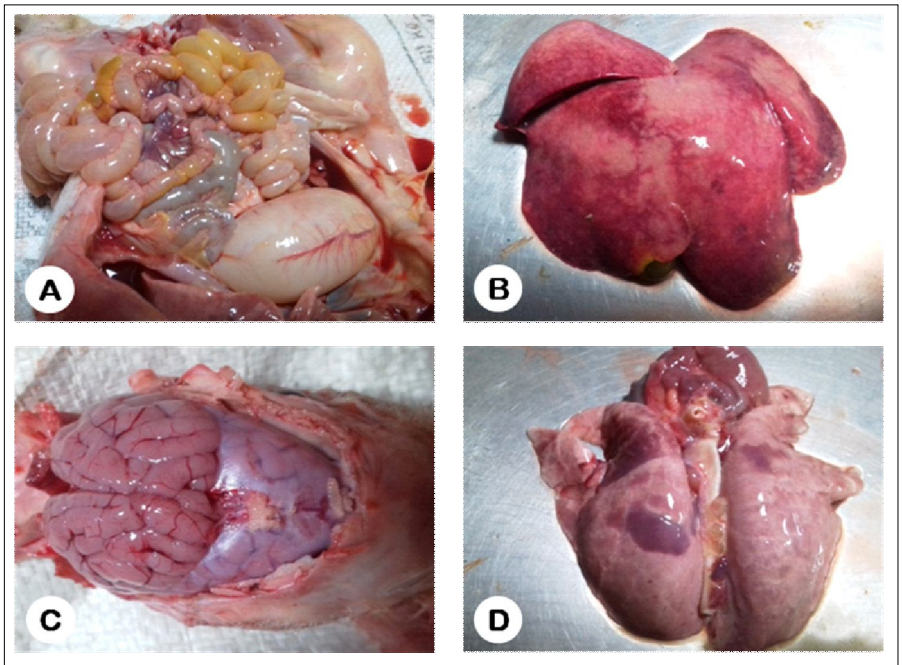
RESULTS AND DISCUSSION

A total of seven out of 78 necropsied animals were found positive for PTV on RT-PCR screening. In RT-PCR, the samples positive for PTV yielded specific amplicons of 321 bp whereas all the other tested samples were found negative for PSV (Fig 1). The RT-PCR positive cases of PTV were confirmed by sequencing and two processed sequences was submitted to the GenBank (Accession nos. MW022462 and MW022463). All the PTV positive cases were less than 2 months of age suggesting the prevalence of the PTV in pre-weaned piglets (Table 1). A total of six out of seven positive cases were presented with the history of diarrhoea indicating PTV as an etiological agent of diarrhoea. A total of seven intestinal contents from necropsied animal were found positive for PTV in RT-PCR screening whereas rest of the tissues included in the study were found negative suggesting no or minimal viral load in these tissues. However, intestinal content of four necropsied animals was found positive for PTV and EV-G both indicating the co-infection of these enteric picornaviruses. Details of necropsied animals positive for PTV are described in Table 1.



Lane 1-5: PTV Positive sample, Lane M: Marker (100bp) and Lane N: Negative test control.

Fig 1: Ethidium bromide stained 1.5% agarose gel showing 321 bp amplicons of PTV.



A: Distended and thin walled intestine filled with yellowish diarrhoeic content; B: Enlarged mottled liver with areas of haemorrhages interspersed with normal areas; C: Moderate degree of congestion of cerebral vessels along with clouding of the meninges; D: Non-collapsible lungs with mild degree of pneumonia.

Fig 2: Gross lesions of PTV in different organs of affected animals.

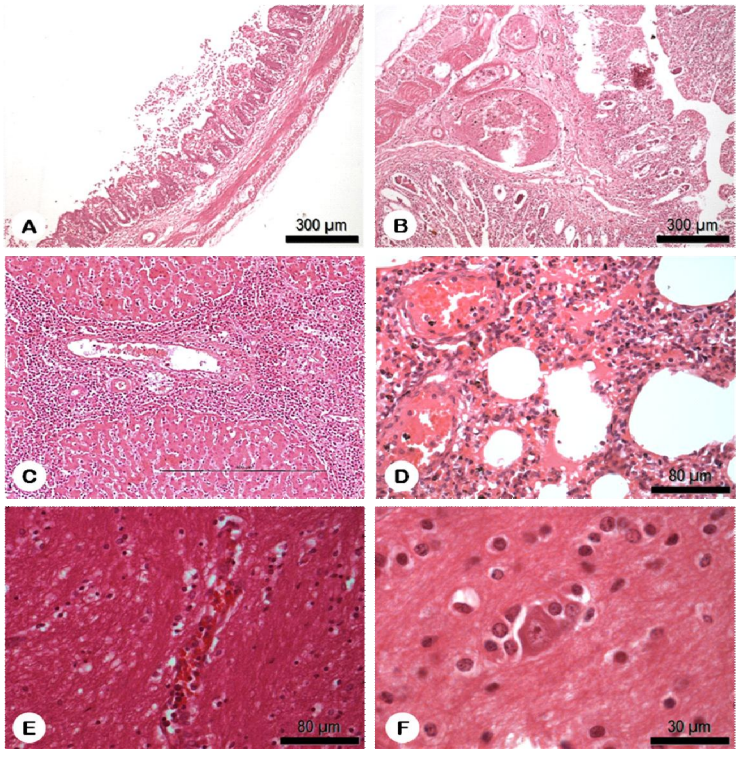
Gross lesions of PTV

The diarrhoeic piglets were severely dehydrated when presented for the post-mortem. On necropsy the intestinal wall was thined and distended with yellowish coloured diarrhoeic content in some cases. Moreover, frequent congestion of serosal and mucosal vessels along with whitish semi-solid to mucoid content was also evident in affected piglets. Gross lesions were observed mostly in the small intestine of the gastrointestinal tract whereas caecum, colon and rectum were found free from gross lesions. In addition, jejunum was observed as the most affected portion of the intestine. Furthermore, the mesenteric lymph nodes of the infected animals (n = 5) were congested. Extra intestinal lesions were observed in brain, liver and lungs (Fig 2). These findings were in line of Yamada *et al*. (2004) and Salles *et al*. (2011).

The gross lesions observed in the lungs affected with PTV ranged from mild to severe degree of pneumonia. The pleura was found thickened and tightly adhered to the non-collapsible lungs in few cases (n = 3). Liver of infected piglets were found enlarged and mottled along with scattered areas of haemorrhages. Petechial haemorrhages over the surface of spleen were also observed. In CNS, lesions ranged from mild to severe congestion of meningeal vessels along with thickening and clouding of meninges. The congestion of vessels of the brain parenchyma was observed as a common finding (Fig 2).

Histopathology

The microscopic lesions associated with PTV infection include severe villous atrophy with desquamation of villous epithelium in duodenum, jejunum and ileum. Additionally, congestion of mucosal vessels, goblet cell hyperplasia along with infiltration of mononuclear cells is the common finding of PTV affected piglets. The mesenteric lymph nodes exhibited mild vascular congestion and depletion of lymphoid cells in the follicles of cortical region along with eosinophilic infiltration. Mild to moderate degree of lymphoid depletion were observed in the Peyer's patches of the ileum. Similar findings were observed by Zhang



A: Complete loss of villous structure and severe desquamation of villi. Jejunum. 100×, H and E; B: Engorgement of submucosal vessels and infiltration of inflammatory cells. Ileum. 100×, H and E; C: Degeneration of hepatocytes and severe infiltration of mononuclear cells in the peri-portal area. Liver. 100×, H and E; D: Severe vascular congestion and infiltration of mononuclear cells in the inter-alveolar septa. Lungs. 400×, H and E; E: Vascular engorgement of brain capillaries along with mild perivascular cuffing. Brain. 400×, H and E; F: Neuronal degeneration evident by satellitosis in the cerebrum. Brain. 1000×, H and E.

Fig 3: Microscopic lesions of PTV in different organs of affected animals.

et al. (2010) in experimentally inoculated pigs in which pronounced loss of villi in small intestine, severe infiltration of mononuclear cells and mucosal degeneration of lamina propria was observed. The CNS lesions include mild to severe degree of meningeal and parenchymal congestion along with perivascular cuffing. Neuronal degeneration, satellitosis and neuronophagia were also observed in PTV affected piglets. Our findings were in line with the other studies on PTV infection in which perivascular cuffs and gliotic foci were observed along with phagocytic vacuoles and axonal degeneration (Yamada *et al*., 2004; Salles *et al*., 2011). The lesions in the lungs consist of mild to severe

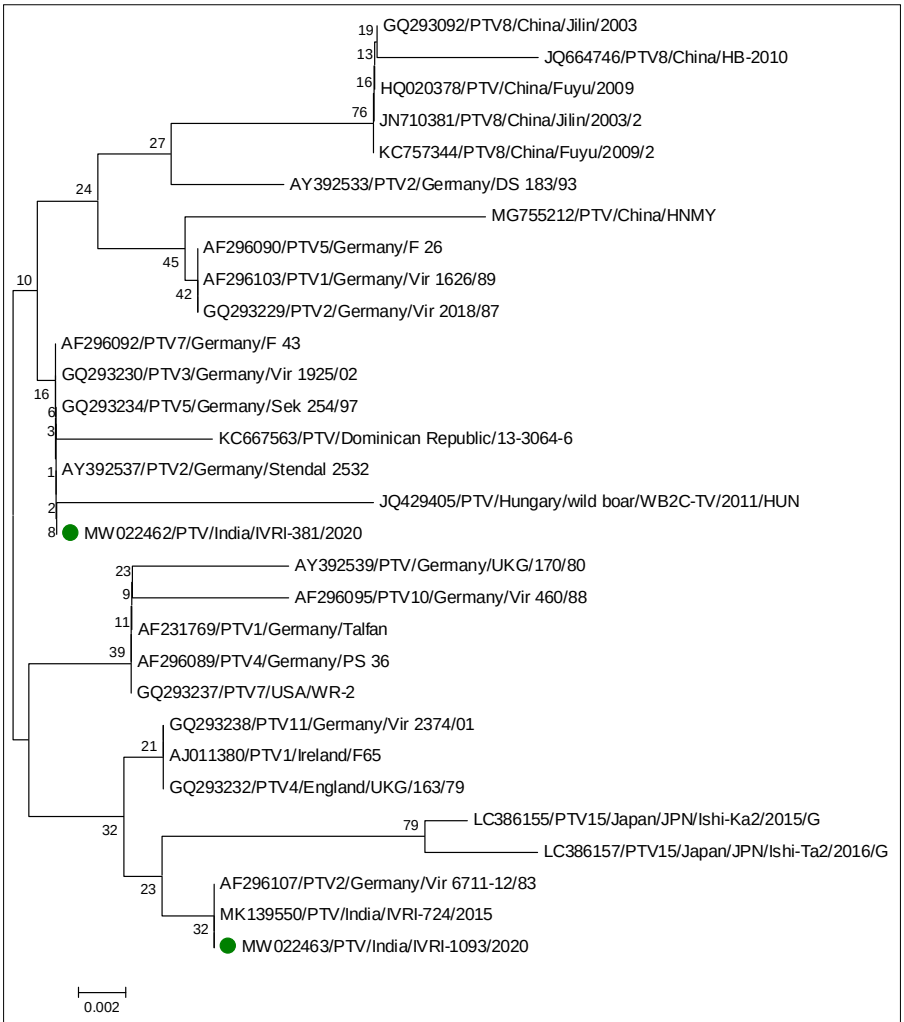


Fig 4: Phylogenetic tree of the Indian isolates of PTV (PTV/India/IVRI-1093/2020; Accession no. MW022463 and PTV/India/IVRI-381/2020; Accession no. MW022462) with reference sequences based on 5′ UTR region of PTV genome using the Neighbor-Joining method.

Table 1: Details of necropsied animals positive for porcine teschovirus in RT-PCR.

Necropsy no.	Breed	Age (Days)	Sex	Type of sample	Virus identified
71 A/19	CB	5 D	F	Intestinal tissue	PTV
364 A/19	CB	17 D	F	Intestinal tissue	PTV
366 A/19	CB	10 D	F	Intestinal tissue	PTV
466 A/19	CB	47 D	F	Intestinal content	PTV, EV-G
346 A/19	CB	9 D	M	Intestinal content	PTV, EV-G
347 A/19	CB	7 D	F	Intestinal content	PTV, EV-G
355 A/19	CB	9 D	M	Intestinal content	PTV, EV-G

degree of bronchopneumonia, interstitial pneumonia or a combination of both. Liver of affected animals showed degeneration of hepatocytes and severe infiltration of mononuclear cells in the peri-portal area (Fig 3). Immunohistochemistry for localization of antigen in the tissues could not be done in this study due to the unavailability of specific antibodies which may be attempted in future studies after raising specific antibodies in suitable animal model. In the present study, only PTV positive cases were considered for the pathological investigation in order to rule out the possible involvement of EV-G in the pathology.

Although, PTV infections are frequently asymptomatic but they have also been found associated with a wide range of illness in pigs ranging from asymptomatic to acute fatal infection including encephalomyelitis, diarrhoea, various reproductive disorders and severe pneumonia (Yamada *et al*., 2004; Knowles, 2006; Ray *et al*., 2020). Our study was found in accordance with the above mentioned studies and further studies are essential in order to establish the pathogenesis of PTV which is warranted for better understanding of the disease.

Genetic characterization of PTV

Phylogenetic analysis based on 5¼ UTR region was done by construction of phylogenetic tree using neighbor-joining method with the bootstrap replicates of 1000. The two isolates of the present study were analysed along with 28 sequences retrieved from NCBI database after construction of phylogenetic tree. The phylogenetic analysis revealed that the isolate PTV/India/IVRI-381/2020 (Accession no. MW022462) clustered with PTV-13 strain wild boar/WB2C-TV/2011/HUN (Accession no. JQ429405) of Hungary whereas the isolate PTV/India/IVRI-1093/2020 (Accession no. MW022463) formed a clade with PTV-2 strain Vir 6711-12/83 (Accession no. AF296107) of Germany (Fig 4). Molecular characterization based on the VP1 region of the viral genome done by Ray *et al*. (2020) in India revealed the circulation of PTV serotype 5 and serotype 8 along with a new variant which showed significant nucleotide diversity from the known serotypes of the teschoviruses.

CONCLUSION AND FUTURE PROSPECTS

The present study concludes that PTV primarily affects nervous system, gastro-intestinal tract and lungs in the pigs. The virus is prevalent in pre-weaned piglets of less than two month of age with significant pathological changes. The PTV is often found associated with large outbreaks of diarrhoea and nervous disorders. The major pathological findings include mild to severe encephalomyelitis, interstitial pneumonia and severe catarrhal enteritis.

This study revealed that the PTV is circulating among the Indian pigs and proper surveillance is crucial to minimize the economic losses attributed to this pathogen. However, further molecular and immunological studies are necessary for development of diagnostics and therapeutics for routine screening and subsequent management of PTV in pigs.

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Conflict of interest

The authors declare that they have no conflict of interest.

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