Archives of Virology (2022) 167:1529–1545
https://doi.org/10.1007/s00705-022-05459-x

ORIGINAL ARTICLE

First report on co-isolation and whole-genomic characterisation of mammalian orthorubulavirus 5 and mammalian orthoreovirus type 3 from domestic pigs in India

Fateh Singh[1] · Katherukamem Rajukumar[1] · Dhanapal Senthilkumar[1] · Govindarajulu Venkatesh[1] · Deepali Srivastava[1] · Subbiah Kombiah[1] · Sandeep Kumar Jhade[1] · Vijendra Pal Singh[1]

Received: 31 December 2021 / Accepted: 12 March 2022 / Published online: 23 May 2022

Abstract

During a surveillance study to monitor porcine epidemic diarrhoea virus and transmissible gastroenteritis virus in India, a total of 1043 swine samples including faeces (n = 264) and clotted blood (n = 779) were collected and tested. Five samples (four faecal and one serum) showed cytopathic effects in Vero cells. Transmission electron microscopy of infectious cell supernatant revealed the presence of two types of virions. Next-generation sequencing (*de novo*) allowed the complete genome sequence of mammalian orthorubulavirus 5 (MRuV5; 15246 bp) and that of all 10 gene segments of mammalian orthoreovirus to be determined. Genetic analysis of MRuV5 revealed grouping of the Indian MRuV5 with isolates from various mammalian species in South Korea and China, sharing more than 99% nucleotide sequence identity. The deduced amino acid sequences of the HN, NP, and F genes of MRuV5 isolates showed three (92L, 111R, 447H), two (86S, 121S), and two (139T, 246T) amino acid substitutions, respectively, compared to previously reported virus strains. Phylogenic analysis based on S1 gene sequences showed the Indian MRV isolates to be clustered in lineage IV of MRV type 3, with the highest nucleotide sequence identity (97.73%) to MRV3 strain ZJ2013, isolated from pigs in China. The protein encoded by the MRV3 S1 gene was found to contain the amino acid residues 198-204NLAIRLP, 249I, 340D, and 419E, which are known to be involved in sialic acid binding and neurotropism. This is the first report of co-isolation and whole-genomic characterisation of MRuV5 and MRV3 in domestic pigs in India. The present study lays a foundation for further surveillance studies and continuous monitoring of the emergence and spread of evolving viruses that might have pathogenic potential in animal and human hosts.

Introduction

The continuous threat of emerging pathogens that cause infectious diseases in humans and animals is a major challenge to the global health care system. A number of novel viruses have emerged in the last few decades, and some of them are able to infect multiple host species, including humans [1, 2]. RNA viruses, which undergo frequent genetic changes, contribute substantially to the occurrence of emerging infectious diseases, sometimes overcoming species barriers to adapt to a new host or ecological niche [3].

Mammalian orthorubulavirus 5 (MRuV5), previously called parainfluenza virus 5 (PIV5), belongs to the genus *Orthorubulavirus* of the family *Paramyxoviridae*. Its genome is a 15,246-nt single-stranded RNA molecule that is packaged in spherical or polymorphous virions that are 50–200 nm in diameter [4]. The MRuV5 genome contains a 3′ leader region, a 5′ trailer region, and seven non-overlapping genes (NP, V/P, M, F, SH, HN, and L) [5]. Due to a specific RNA editing mechanism, the V/P gene encodes two distinct structural proteins, V and P, and thus seven genes of the MRuV5 genome encode a total of eight proteins [6]. MRuV5 has been isolated from various host species, including humans, pigs, dogs, cattle, cats, hamsters, guinea pigs, and lesser pandas [6–9]. It has often been reported to cause asymptomatic respiratory infections in many mammalian species [8]. Moreover, MRuV5 strains have been isolated from intestinal tissues of diarrhea-affected piglets and may contribute to enteric disease in pigs [10].

Handling Editor: Ana Cristina Bratanich.

✉ Fateh Singh
fateh.ars07@gmail.com

[1] ICAR-National Institute of High Security Animal Diseases, Bhopal, Madhya Pradesh 462022, India

Mammalian orthoreovirus (MRV), a member of the genus *Orthoreovirus* of the family *Spinareoviridae,* is a non-enveloped double-stranded RNA virus with a double-capsid structure containing 10 genomic RNA segments [11]. These viral RNA segments include three large (L), three medium (M), and four small (S) segments, based on electrophoretic mobility [12]. Due to its segmented genome, genetic recombination among segments of reoviruses is possible, and reassortant strains are detected frequently [13, 14].

The inner capsid of the MRV virion is formed by the proteins λ-1 and σ-2, while the outer capsid is made up of the proteins λ-2, μ-1, σ-3, and σ-1 [15]. The σ-1 protein, which is located in the outer capsid of the virion [16, 17], is encoded by gene segment S1 [18]. The σ-1 protein facilitates haemagglutination [19], attachment of the virus to cellular receptors [20, 21], and serotype-specific neutralisation [22]. There are four known serotypes of MRV based on their sequence and the antigenicity of the σ-1 protein. These serotypes include prototype strain type 1 (Lang, T1L), type 2 (Jones, T2J), type 3 (Dearing, T3D), and type 4 (Ndelle, T4N) [23]. Based on S1 gene phylogeny, MRV type 3 strains can be classified into four lineages, and most of the Asian MRV3 strains cluster into lineage IV [24].

Like MRuV5, MRV has a wide host range and infects multiple mammalian species, including pigs, dogs, cattle, sheep, horses, cats, mice, humans, and non-human primates [25]. Previously, MRV was thought to cause only asymptomatic or mild respiratory and enteric infections [25]. However, recently, MRV has been found to cause several clinical infections in humans and other mammalian species. Clinical cases of enteritis, acute respiratory infections, and encephalitis in humans due to reassortant MRV strains have increased worldwide, and the causative MRV strains have animal reservoirs [26, 27]. MRV2 and 3 are known to cause respiratory infections, encephalitis, and diarrhoea in humans [26, 27], while MRV3, alone or with other pathogens, has been reported in association with enteric disease in pigs from Asia, North America, and Europe [24, 28–31]. Owing to the confluence of environmental, socio-economic, and demographic factors, tropical regions such as India are vulnerable 'hotspots' for the emergence of infectious diseases [32, 33]. Due to a lack of species barriers, the prime concern is zoonotic transmission and spread of MRuV5 and MRV in humans and animals. Surveillance studies targeting swine populations might be an important strategy to detect emerging and novel viral pathogens, as pigs act as reservoirs of a diverse range of viruses. Identification of emerging or exotic viruses provides valuable information on upcoming potential threats and can aid in providing disease alerts for necessary preparedness. In this paper, we report the co-isolation and whole-genomic characterisation of MRuV5 and MRV type 3 recorded for the first time in domestic pigs in India.

Materials and methods

Samples

During a surveillance study to monitor emerging and exotic swine enteric viruses such as porcine epidemic diarrhoea virus (PEDV) and transmissible gastroenteritis virus (TGEV), a total of 1043 samples, including faeces (n = 264) and clotted blood (n = 779), were randomly collected from pigs, irrespective of age and any clinical enteric disease, from backyard and commercial farms located in different states in India during the period from 2017 to 2019. The sampling details are shown in Table 1. Faecal samples in the form of rectal swabs were collected in 1 ml of sterile phosphate-buffered saline (PBS) containing 2× antibiotics and anti-mycotic solution (Sigma-Aldrich, USA). All samples were transported under refrigerated conditions to the biosafety level 3 (BSL-3) laboratory at ICAR-National Institute of High Security Animal Diseases, Bhopal, for testing.

Sample processing

Serum was separated from the clotted blood by centrifugation at 1200 *g* for 15 min. Faecal swab samples were suspended in sterile PBS to make a 20% (w/v) homogenate. Supernatants of faecal homogenates were collected after centrifugation at 8000 *g* for 15 min. The serum and faecal supernatants were kept in two sets: one set to extract the viral

Table 1 Details about the samples included in the present study

State	District	Faecal swabs	Clotted blood
Mizoram	Champhai	88[§]	177[§]
	Aizawl	40[*]	42[§] + 115[*]
	Serchhip	13[*] + 12[§]	13[*] + 52[§]
Assam	Kamrup	20[*] + 56[§]	20[*] + 53[§]
Chhattisgarh	Rajnandgaon	24[§]	24[§]
Madhya Pradesh	Jabalpur	11[*]	11[*]
	Indore	–	20[¥]
	Dhar	–	44[¥]
	Bhopal	–	10[¥]
	Gwalior	–	10[¥]
	Shivpuri	–	10[¥]
	Narsinghpur	–	08[¥]
West Bengal	Darjeeling	–	88[¥]
	Jalpaiguri	–	62[¥]
Maharashtra	Pune	–	20[¥]
Total		264	779

[§]Samples collected from backyard pigs; [*]Samples collected from commercial pig farms;

[¥]Samples received at the laboratory for disease diagnosis

RNA and the other for virus isolation. For virus isolation, the faecal supernatants were filtered through 0.22-µm-pore-sized syringe filters for decontamination.

Screening of samples by reverse transcription PCR (RT-PCR)

RNA was extracted from faecal supernatants and serum samples using a QIAamp Viral RNA Extraction Kit (QIAGEN, Hilden, Germany). One-step RT-PCR was carried out for the detection of the genomes of PEDV and TGEV as described previously, using virus-specific primers [34] (Table 2) and an Access RT-PCR System (Promega Corporation, Madison, USA). RT-PCR products were analysed by agarose gel electrophoresis.

Virus isolation

For virus isolation, dilutions (1:5 and 1:10) of the faecal supernatants and clarified serum samples were inoculated in duplicate into sub-confluent monolayers of Vero cells grown in Eagle's minimum essential medium (EMEM) in 96-well cell culture plates. Briefly, 10 µl of faecal supernatant or 5 µl of clarified serum was inoculated onto the cells of each well of a cell culture plate containing 40 µl and 45 µl, respectively, of plain EMEM containing trypsin at a concentration of 10 µg/ml, after which the plate was incubated at 37 ºC for 1 h for virus adsorption. After virus adsorption, 50 µl of plain EMEM containing trypsin at a concentration of 10 µg/ml was added to adjust the final volume in each well to 100 µl. The uninoculated wells containing only plain EMEM served as negative controls. The plates were incubated at 37 ºC for 5-6 days in an incubator with 5% CO_2. The cells were observed daily using an inverted microscope for the development of a cytopathic effect (CPE). Subsequently, two more serial passages were done using 1:5-diluted frozen and thawed cell culture supernatants from the previous passage. At the end of the third passage, the cell supernatants were tested for the presence of PEDV and TGEV genomic RNA by RT-PCR.

Table 2 Primers used for screening and specific detection of swine viruses

Virus/ gene	Primers	Amplicon size (bp)	Annealing temp (°C)	Reference
Primers used for screening				
PEDV M gene	5'-GGGCGCCTGTATAGAGTTTA-3' 5'-AGACCACCAAGAATGTGTCC-3'	412	55	[34]
TGEV N gene	5'-GATGGCGACCAGATAGAAGT-3' 5'-GCAATAGGGTTGCTTGTACC-3'	612	55	[34]
PRV VP4	F-5'-TGGCTTCGCCATTTTATAGACA-3' R-5'-ATTTCGGACCATTTATAACC-3'	876	50	[35]
Enterovirus	F-5'-GTACCYTTGTRCGCCTGTT-3' R-5'-ATTGTCACCATAAGCAGCCA-3'	536	52	[36]
Sapelovirus (PEV8)	F-5'-CTTAAGGTGGTTGTATCCCCTAC-3' F-5'-TTCAACTGACTATACTAGTTACAGG-3'	413	52	[37]
PPV	F-5'-ACACGCATCAAGACTCATAC-3' R-5'-TCACTGTGTAGTCCTGTTTG-3'	531	56	[38]
Primers used for specific detection of MRuV5 and MRV				
MRuV5-NP	F- 5'-ATGTCATCCGTGCTTAAAGC-3' R- 5'-CTAGATGTCAAGATCACCCA-3'	1530	50	[6]
MRuV5-P	F- 5'-ATGGATCCCACTGATCTGAG-3' R- 5'-TCAAATTGCACTGCGGATGA-3'	1177	50	[6]
MRuV5-F	F1- 5'-AAATCATATTAAGACTAT-3' R1- 5'-GTCCTATCGTTTTTTTCTTA-3'	1749	50	[10]
MRuV5-M	F- 5'-ATGCCATCCATCAGCATCCC-3' R- 5'-TCATTCCAGCTCCGTCAGGT-3'	1134	50	[6]
MRV L1	F1-5'-GCATCCATTGTAAATGACGAGTCTG-3' R1-5'-CTTGAGATTAGCTCTAGCATCTTCTG-3'	416	50	[40]
MRV L1	F2 5'-GCTAGGCCGATATCGGGAATGCAG-3' R2 5'-GTCTCACTATTCACCTTACCAGCAG-3'	344	50	[40]
MRV L3	F1 5'-CAGGATGAAGCGGATTCCAA-3' R1 5'-GGATGATTCTGCCATGAGCT-3'	696	50	[41]

PEDV, porcine epidemic diarrhoea virus; TGEV, transmissible gastroenteritis virus; PRV, porcine rotavirus; PEV, porcine enterovirus; PPV, porcine parvovirus; MRuV5, mammalian orthorubulavirus 5; MRV, mammalian orthoreovirus

Screening of cell culture supernatants by RT-PCR/PCR

Cells exhibiting CPE that tested negative for PEDV and TGEV were further investigated for the presence of other swine enteric viruses, including porcine rotavirus (PRV) [35], porcine enterovirus (PEV) [36], porcine sapelovirus (PSV) [37], and porcine parvovirus (PPV) [38].

Viral RNA and DNA were extracted from the CPE-positive as well as uninfected control cell culture supernatants using a QIAamp Viral RNA Extraction Kit (QIAGEN, Hilden, Germany) and a QIAamp Viral DNA Extraction Kit (QIAGEN, Hilden, Germany), respectively. The nucleic acid was used as a template for RT-PCR or PCR, as appropriate, using virus-specific primers and methods described previously (Table 2).

Haemagglutination test

Cell culture supernatants from CPE-positive wells were also tested for haemagglutination (HA) activity using pig, guinea pig, and chicken red blood cells (RBCs) as per the standard protocol [39].

Ultracentrifugation

The CPE-positive cell culture supernatants were processed for ultracentrifugation to concentrate the virus particles. The cell suspensions after three freeze-thaw cycles were clarified by centrifugation at 8000 *g* for 15 min at 4 ºC. The supernatants were overlaid on one-third volume of 20% sucrose (w/v, prepared in TNE buffer) in ultracentrifuge tubes. Ultracentrifugation was carried out at 150,000 *g* and 4 ºC for 2 h in a Sorvall WX 90 ultracentrifuge with a swinging bucket rotor (Thermo Fisher Scientific, Waltham, MA, USA). After ultracentrifugation, the supernatants were discarded and the sediment at the bottom of the tube was resuspended in 200 µl of autoclaved distilled water.

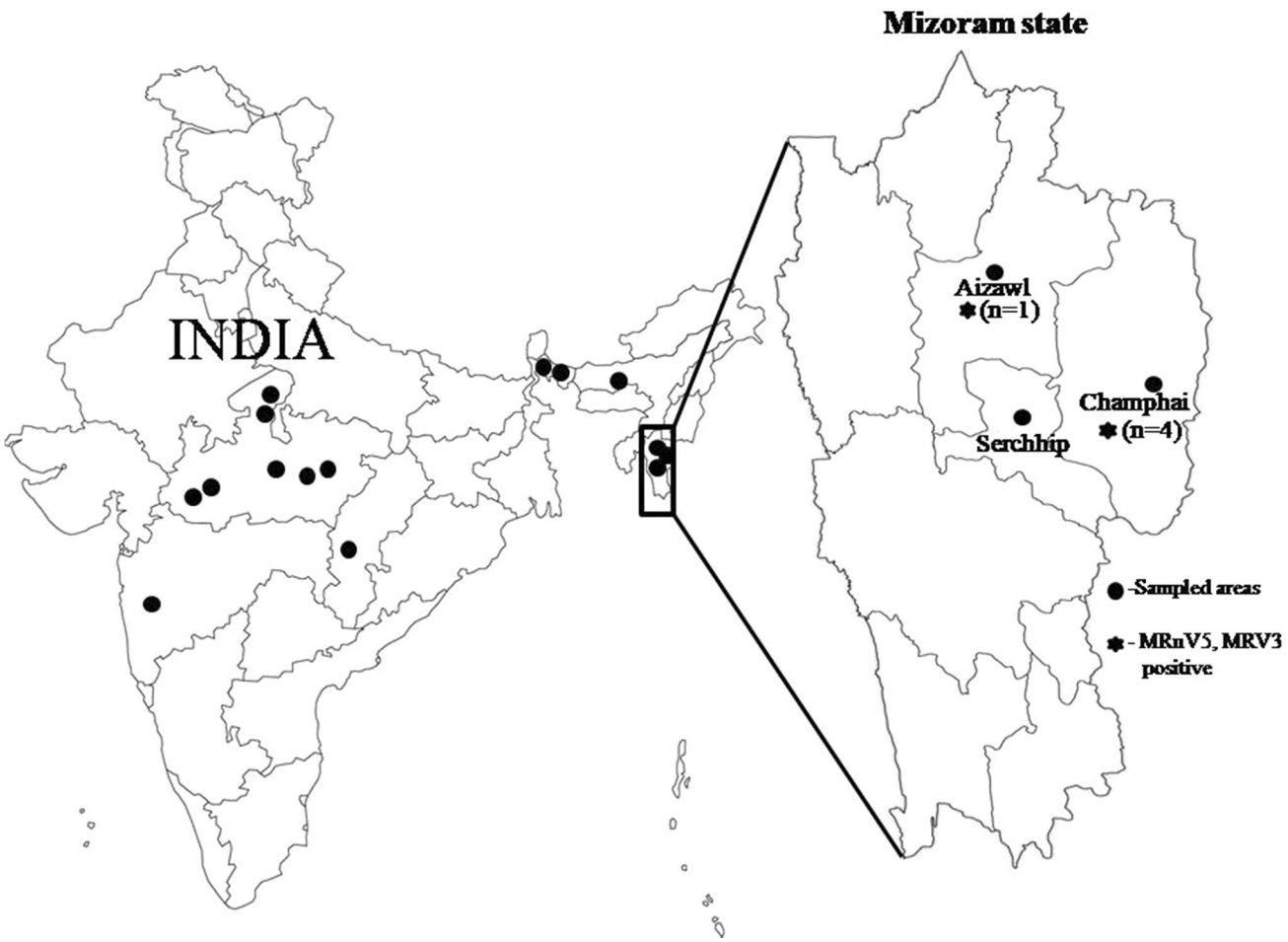

Fig. 1 Geographical locations where sampling was carried out and MRuV5- and MRV3-positive areas. Locations are shown at the district level.

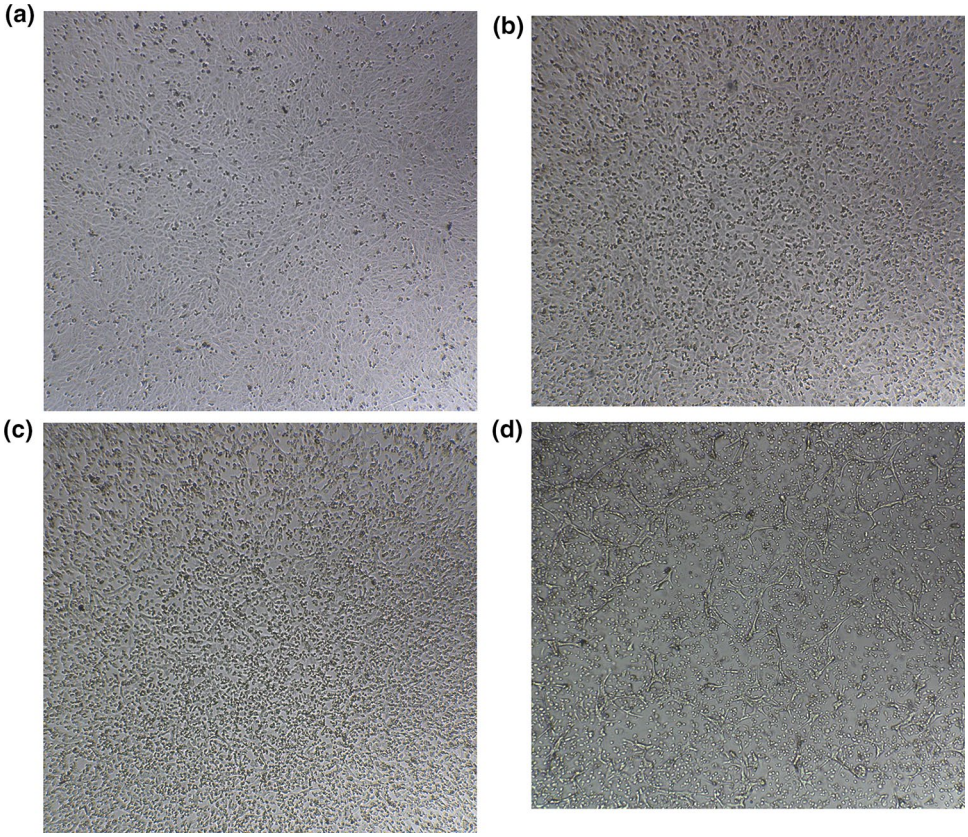

Fig. 2 Cytopathic effect (CPE) in Vero cells infected with MRuV5 and MRV3. (a) Uninoculated cells. (b) Rounding and clumping of cells (48 hpi). (c) Moderate CPE with cell detachment (72 hpi). (d) Severe CPE with more cell detachment (96 hpi)

Transmission electron microscopy

For electron microscopy, a drop of the ultracentrifuged suspension was allowed to adsorb onto a formvar-carbon–coated copper grid for 3-5 min, and the adsorbed material was negatively stained with 2% phosphotungstic acid for 45 s. The stained grids were air-dried and viewed in a transmission electron microscope (Talos, FEI, Hillsboro, OR, USA).

Next-generation sequencing

RNA was extracted from the ultracentrifuged cell culture supernatant of one faecal sample (3013814) and one serum sample (3013789) and subjected to next-generation sequencing (*de novo*) using a NextSeq500 platform (Illumina) commercially at Eurofins Genomics India Pvt. Ltd., Bengaluru. Raw sequence data were processed to obtain high-quality clean reads using Trimmomatic v0.38. The resulting reads were aligned to the hg 19 human reference genome sequence using BWA MEM (0.7.17). Unmapped reads were identified using Samtools, and *de novo* assembly of unmapped reads was performed using CLC Workbench 9.0. A BLASTn search was performed using the longest contigs to identify viral sequences. Two contigs with similarity to viral sequences were subjected to gene prediction using the GeneMarkS tool, and the predicted genes were annotated using the Diamond tool (BLASTx).

Phylogenetic analysis

The sequence data were assembled and analysed with respect to the reference sequences available in the NCBI GenBank database. The whole genome sequence of MRuV5 and the segment sequences of MRV were used to perform phylogenetic analysis. The MRV serotype was determined based on genetic analysis of the S1 segment sequence. The evolutionary history of the virus was inferred using the maximum-likelihood method and the Tamura-Nei model available in MEGA X software (https://www.megasoftware.net/).

Detection of specific genes of MRuV5 and MRV

MRuV5 and MRV were also identified by one-step RT-PCR and sequencing. Previously published primers specific for the nucleoprotein (NP), fusion (F) protein, phosphoprotein (P), and membrane (M) protein genes were used to verify the presence of MRuV5, while primers specific for the L1 and L3 segments were used to detect the MRV in Vero cell supernatants of the four faecal samples and the one serum sample that produced CPE. RT-PCR amplification was performed by adding 4 µl of AMV/ *Tfl* 5× reaction buffer, 0.2

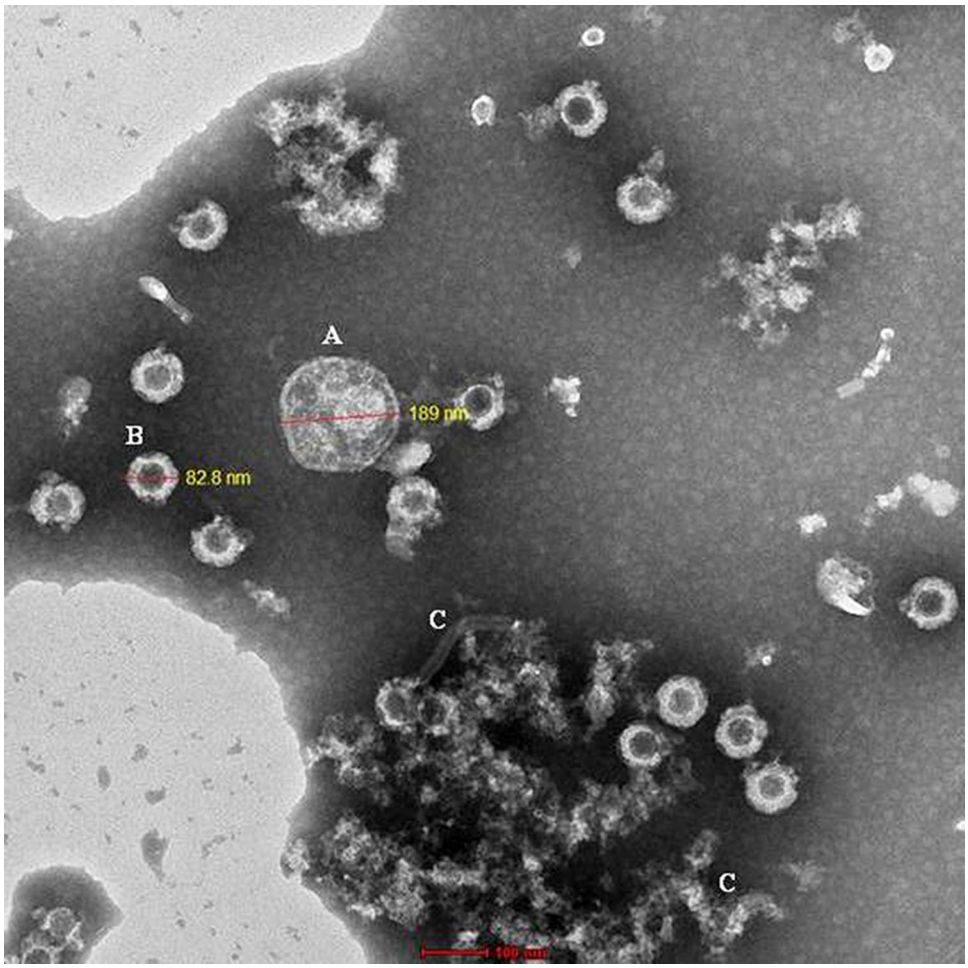

Fig. 3 Transmission electron micrograph of the supernatants of the infected Vero cells using negative staining with 2% sodium phosphotungstate. The micrograph shows a round enveloped paramyxovirus (MRuV5) virion (A), an icosahedral nonenveloped reovirus virion (MRV3) (B), and the ultrastructure (nucleocapsid core) of MRuV5 (C). (Scale bar = 100 nm).

mM dNTPs, 0.75 mM $MgSO_4$, AMV reverse transcriptase (2 U), and *Tfl* DNA polymerase (2 U), using an Access RT-PCR System (Promega Corporation, Madison, USA), with 10 picomoles of each primer and 2 µl of RNA template for 20-µl amplification reactions. One-step RT-PCR conditions included reverse transcription of RNA at 45 ºC for 30 min followed by initial denaturation at 95 ºC for 3 min and 35 cycles of denaturation at 95 ºC for 20 s, annealing at 50 ºC for 45 s, and extension at 72 ºC for 30 s to 1.5 min (30 s for the L1 and L3 segments of MRV, 1 min for the M and P genes of MRuV5, and 1.5 min for the NP and F genes of MRuV5), and a final extension at 72 ºC for 5 min. PCR products were analysed by agarose gel electrophoresis. The primer details [6, 10, 40, 41] are provided in Table 2.

The PCR products were gel-purified using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions and subjected to nucleotide sequencing by the Sanger method for confirmation.

Results

All of the samples were negative for PEDV and TGEV genomic RNA by RT-PCR. Five samples, including one faecal swab (lab accession no. 3013772) collected from a commercial pig farm located in the Aizawl district of the state of Mizoram, one serum sample (lab accession no. 3013789) collected from a backyard pig in the town of Champhai of the Champhai district of the state of Mizoram, and three faecal swabs (lab accession no. 3013814, 3013820, and 3013823) collected from backyard pigs in the village of Zokhawthar located in the India-Myanmar border area of Champhai district (Fig. 1), showed CPE in Vero cells by the third passage. The CPE, characterised by rounding and detachment of cells, started at 48 h postinfection (hpi), and by 72 hpi, more than 50% of the cells were detached (Fig. 2). About 80% of the cells were detached by 96-120 hpi, after which the cells were frozen.

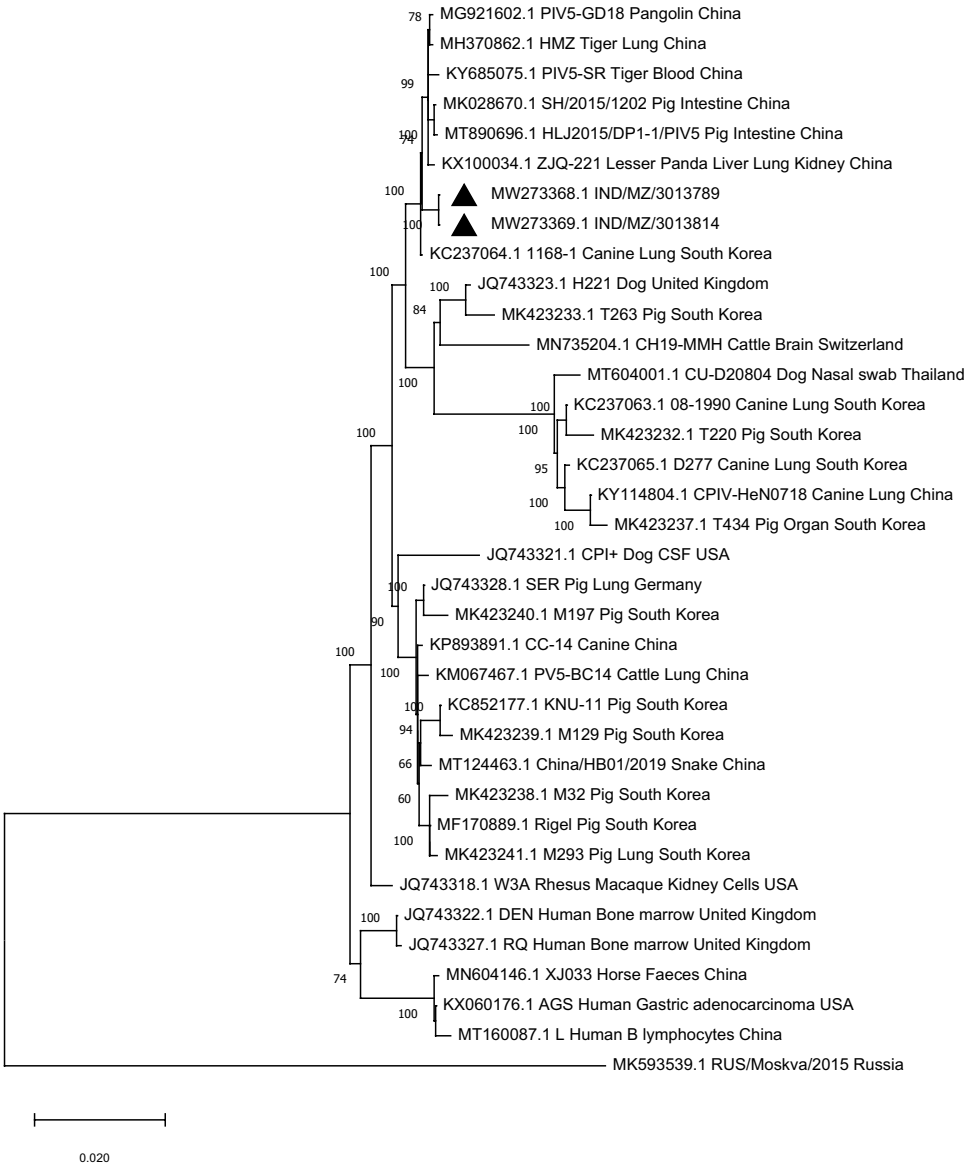

Fig. 4 Phylogenetic analysis of MRuV5 based on the whole genome sequence. The phylogenetic tree was constructed using MEGA X software, by the Construct/Test maximum-likelihood method with the Tamura-Nei model and 1000 bootstrap replications. Bootstrap values ≥60% are shown. The dark triangles indicate isolates from the present study.

The CPE-positive cell culture supernatants were found to be negative for PEDV, TGEV, PRV, PEV, PSV, and PPV when tested by RT-PCR/ PCR. The cell culture supernatants of CPE-producing samples showed the ability to cause haemagglutination (HA) of pig and guinea pig RBCs. No HA activity was observed with chicken RBCs.

TEM analysis showed the presence of two types of viruses: The first type had pleomorphic enveloped virions with a diameter ranging from 99 nm to 261 nm (typical diameter range, 150-200 nm) resembling a paramyxovirus (Fig. 3). A characteristic 'herringbone-like' nucleocapsid of about 17 nm in diameter could be seen outside the disrupted virus particles. The second type appeared to be icosahedral, non-enveloped virus particles ranging from 68 to 89 nm in diameter (typical diameter range, 78-85 nm), resembling a reovirus (Fig. 3).

Next-generation sequencing generated 828 mb and 476 mb of data for sample number 3013789 and sample number 3013814, respectively. BLASTn analysis of the longest scaffolds showed the top hits for both the samples to be mammalian orthorubulavirus 5 (MRuV5) and mammalian orthoreovirus (MRV). Sequence analysis showed the presence of seven genes encoding a nucleocapsid protein (NP) (1530 nucleotides, 509 amino acids), a V protein (669 nucleotides, 222 amino acids), a membrane (M) protein (1134 nucleotides, 377 amino acids), a fusion (F) protein (1656 nucleotides, 551 amino acids), a small hydrophobic (SH) protein (135 nucleotides, 44 amino acids), a haemagglutinin-neuraminidase (HN) (1698 nucleotides, 565 amino acids), and a large (L) protein (6735 nucleotides, 2244 amino acids)

Table 3 Comparison of MRuV5 strains IND/MZ/3013789 and IND/MZ/3013814 with reported strains

Gene	MRuV5 strain	GenBank accession number	Nucleotide sequence identity	Amino acid sequence identity	Host	Country
NP	PIV5-GD18	MG921602.1	99.74	99.61	Pangolin	China
	SH/2015/1202	MK028670.1	99.67	99.41	Pig	China
	M32	MK423238.1	98.37	97.45	Pig	South Korea
	T263	MK423233.1	97.71	97.25	Pig	South Korea
	T434	MK423237.1	96.41	97.45	Pig	South Korea
	RUS/Moskva/2015	MK593539.1	87.52	93.91	Vero cells*	Russia
V	PIV5-GD18	MG921602.1	99.85	99.55	Pangolin	China
	CU-D399	MT604025.1	99.7	99.55	Dog	Thailand
	Rigel	MF170889.1	98.51	97.75	Pig	South Korea
	CH19-MMH	MN735204.1	97.91	98.65	Cattle	Switzerland
	T434	MK423237.1	96.86	96.4	Pig	South Korea
	RUS/Moskva/2015	MK593539.1	87.89	96.4	Vero cells*	Russia
M	1168-1	KC237064.1	99.82	99.73	Canine	South Korea
	ZJQ-221	KX100034.1	99.65	99.47	Lesser panda	China
	SH/2015/1202	MK028670.1	99.56	99.2	Pig	China
	XJ033	MN604146.1	97.27	97.35	Horse	China
	T434	MK423237.1	96.12	97.08	Pig	South Korea
	RUS/Moskva/2015	MK593539.1	86.95	95.38	Vero cells*	Russia
F	1168-1	KC237064.1	99.76	99.46	Canine	South Korea
	CU-D399	MT604022.1	99.7	99.64	Dog	Thailand
	SH/2015/1202	MK028670.1	99.64	99.09	Pig	China
	T263	MK423233.1	97.52	97.28	Pig	South Korea
	T434	MK423237.1	95.89	95.28	Pig	South Korea
	RUS/Moskva/2015	MK593539.1	85.27	94.01	Vero cells*	Russia
SH	SH/2015/1202	MK028670.1	100	100	Pig	China
	PIV5-GD18	MG921602.1	100	100	Pangolin	China
	T263	MK423233.1	97.04	95.45	Pig	South Korea
	CH19-MMH	MN735204.1	92.42	88.64	Cattle	Switzerland
	Rigel	MF170889.1	85.38	72.09	Pig	South Korea
	M32	MK423238.1	84.62	72.09	Pig	South Korea
HN	CU-D399	MT604023.1	99.59	99.29	Dog	Thailand
	PIV5-GD18	MG921602.1	99.29	98.58	Pangolin	China
	M32	MK423238.1	98.65	97.35	Pig	South Korea
	CH19-MMH	MN735204.1	97.59	97.35	Cattle	Switzerland
	T434	MK423237.1	97.35	97.17	Pig	South Korea
	RUS/Moskva/2015	MK593539.1	85.51	92.57	Vero cells*	Russia
L	1168-1	KC237064.1	99.87	99.82	Canine	South Korea
	PIV5-GD18	MG921602.1	99.85	99.82	Pangolin	China
	HMZ	MH370862.1	99.82	99.73	Tiger	China
	XJ033	MN604146.1	98.07	98.4	Horse	China
	T434	MK423237.1	97.83	98.4	Pig	South Korea
	RUS/Moskva/2015	MK593539.1	88.15	97.64	Vero cells*	Russia

*Vero cells exhibited a cytopathic effect and were hypothesised to have been contaminated by a researcher with respiratory symptoms

in the MRuV5 genome. The whole genome size of MRuV5 was estimated to be 15,246 nt. The whole genome sequences of MRuV5 obtained in this study have been submitted to the NCBI GenBank database (accession numbers: MW273368.1 [IND/MZ/3013789] and MW273369.1 [IND/MZ/3013814]). A gene prediction analysis also showed the presence of MRV with a segmented genome consisting of three large (L1, L2, L3), three medium (M1, M2, M3), and four small

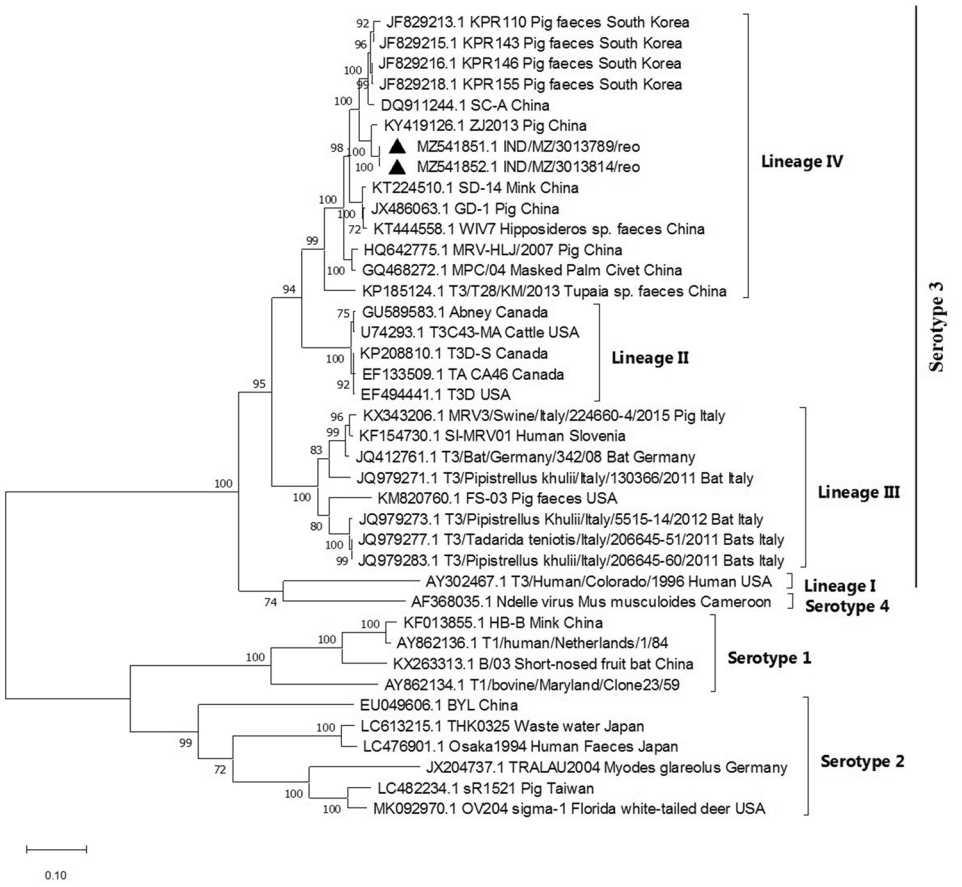

Fig. 5 Phylogenetic analysis of the MRV3 S1 gene. The phylogenetic tree was constructed using MEGA X software, by the Construct/Test maximum-likelihood method, using the Tamura-Nei model and 1000 bootstrap replications. Bootstrap values ≥60% are shown. The dark triangles indicate isolates from the present study.

(S1, S2, S3, S4) segments, and genome lengths of 22,219 bp and 20,512 bp were determined for the IND/MZ/3013789/reo and IND/MZ/3013814/reo isolate, respectively. The difference in genome length of the MRV isolates was due to incomplete sequencing of the L2 segment of the IND/MZ/3013814/reo isolate. The nucleotide sequences of the genome segments of the MRV (IND/MZ/3013789/reo and IND/MZ/3013814/reo) isolates from this study have been submitted to the NCBI GenBank database under accession numbers MZ516371.1 to MZ516381.1 and MZ541850.1 to MZ541858.1.

Whole-genome-based sequence and phylogenetic analysis of the MRuV5 isolate from pigs from the present study showed grouping of the Indian MRuV5 isolate with isolates from various mammalian species in South Korea and China, with more than 99% nucleotide sequence identity (Fig. 4). Nucleotide and deduced amino acid sequence analysis of all seven individual ORFs (NP, V/P, M, F, SH, HN and L) showed > 99% identity to several strains of MRuV5 reported in China, South Korea, and Thailand (Table 3).

The deduced amino acid sequences of the HN, NP, and F gene coding regions showed the presence of three (92L, 111R, 447H), two (86S, 121S), and two (139T, 246T) unique amino acid substitutions, respectively, as compared to previously reported strains of the virus. The predicted amino acid sequence of the HN protein contained the motif QDHVS (186–190) at the receptor-binding site and the residues E390 and Y523 at the cleavage site. The predicted amino acid residues in the HN stalk region included S60, Y77, L90, E91, and Q102, similar to other strains of MRuV5 reported previously. Amino acid residues predicted to be involved in the induction of neutralizing antibodies included as E37, Q342, T437, and F457. Amino acid residues predicted to be involved in host preference were I22, A49, R57, T254, N318, and K460 in the HN protein and T3, S19, T438, L498, S530, and R536 in the fusion protein. The membrane protein showed the presence of one unique amino acid (184L) substitution compared to previously reported strains of the virus.

Phylogenetic analysis based on the S1 gene resulted in clustering of the present MRV isolates in lineage IV of the MRV type 3 serotype (Dearing, T3D), showing the highest nucleotide sequence identity (97.73%) and deduced amino acid sequence identity (96.7%) to MRV3 strain ZJ2013 (KY419126.1), which was isolated from pigs in China (Fig. 5). The nucleotide and deduced amino acid sequence identity values for all gene segments of MRV with respect to some previously reported strains are shown in Table 4.

Table 4 Comparison of MRV strains IND/MZ/3013789/reo and IND/MZ/3013814/reo with reported strains

Gene	MRV strain	Serotype	GenBank accession number	Nucleotide sequence identity	Amino acid sequence identity	Host	Country
L1	WIV8	1	KT444562.1	98.29	99.17	Bat	China
	ZJ2013	3	KY419120.1	95.87	98.82	Pig	China
	SI-MRV02	3	MG457078.1	90.9	98.23	Bat	Slovenia
	BYD1	2	DQ664184.1	88.94	98.1	Human	China
	SI-MRV03	1	MG457088.1	81.52	95.53	Bat	Slovenia
L2	WIV7	3	KT444553.1	97.38	99.32	Bat	China
	HB-A	1	KC462150.1	93.88	99.1	Mink	China
	BYD1	2	DQ664185.1	81.12	96.2	Human	China
	SI-MRV02	3	MG457079.1	76.7	92.84	Bat	Slovenia
	SI-MRV03	1	MG457089.1	74.77	91.4	Bat	Slovenia
L3	Osaka1994	2	LC476897.1	97.13	99.29	Human	Japan
	THK0617	1	LC613221.1	96.55	99.41	Wastewater	Japan
	WIV7	3	KT444554.1	94.91	99.17	Bat	China
	BYD1	2	DQ664186.1	83.58	97.3	Human	China
	SI-MRV03	1	MG457090.1	79.85	96.13	Bat	Slovenia
M1	ZJ2013	3	KY419123.1	96.92	97.33	Pig	China
	HB-A	1	KC462152.1	95.21	95.3	Mink	China
	BYD1	2	DQ664187.1	89.15	92.06	Human	China
	SI-MRV02	3	MG457081.1	86.89	93.48	Bat	Slovenia
	SI-MRV03	1	MG457091.1	79.82	87.88	Bat	Slovenia
M2	Osaka1994	2	LC476899.1	97.6	99.16	Human	Japan
	HB-A	1	KC462153.1	95.02	99.17	Mink	China
	ZJ2013	3	KY419124.1	94.69	99.16	Pig	China
	BYD1	2	DQ664188.1	89.05	98.12	Human	China
	96	2	LC533918.1	83.38	96.67	Pig	Zambia
M3	B/03	1	KX263312.1	96.35	98.09	Fruit bat	China
	ZJ2013	3	KY419125.1	95.61	97.88	Pig	China
	SI-MRV02	3	MG457083.1	90.35	96.62	Bat	Slovenia
	BYD1	2	DQ664189.1	83.94	91.39	Human	China
	MRV2/Swine/Italy/90178-3/2018_M3	2	MT151669.1	82.2	90.4	Pig	Italy
S1	ZJ2013	3	KY419126.1	97.73	95.16	Pig	China
	KPR146	3	JF829216.1	94.89	93.27	Pig	South Korea
	WIV7	3	KT444558.1	92.99	89.1	Bat	China
	SI-MRV02	3	MG457084.1	80.22	83.55	Bat	Slovenia
	BM-100	3	KM820750.1	78.41	78.34	Pig	USA
S2	WIV7	3	KT444559.1	98.73	97.84	Bat	China
	Osaka2005	2	LC476912.1	98.57	98.2	Human	Japan
	BM-100	3	KM820751.1	91.96	96.43	Pig	USA
	BYD1	2	DQ664190.1	84.73	96.11	Human	China
	SI-MRV03	1	MG457095.1	80.40	93.62	Bat	Slovenia
S3	Osaka2014	2	LC476923.1	97.09	98.81	Human	Japan
	HB-A	1	KC462157.1	95.73	96.44	Mink	China
	SI-MRV02	3	MG457086.1	89.46	97.24	Bat	Slovenia
	BYD1	2	DQ664191.1	82.53	92.58	Human	China
	SI-MRV08	2	MT518192.1	74.67	79.15	Human	Slovenia

Table 4 (continued)

Gene	MRV strain	Serotype	GenBank accession number	Nucleotide sequence identity	Amino acid sequence identity	Host	Country
S4	ZJ2013	3	KY419129.1	97.63	98.84	Pig	China
	HB-A	1	KC462158.1	96.81	98.08	Mink	China
	Osaka1994	2	LC476904.1	93.99	97.68	Human	Japan
	BYD1	2	DQ318037.2	85.06	94.59	Human	China
	MRV3/Pig/4476/USA/2014	3	MN233067.1	76.47	83.72	Pig	USA

Phylogenetic trees based on the segments L1, L2, L3, M1, M2, M3, S2, S3, and S4 of MRV3 are shown in Figure 6.

The deduced amino acid sequence of the σ-1 protein, encoded by the S1 segment, showed changes in 15 amino acids compared to MRV3 strain ZJ2013. The MRV strains reported in the present study showed three unique amino acid changes (V49, H65, and I186), which are distinct from most of the strains reported so far. Further analysis of the deduced amino acid sequence of the σ-1 protein revealed that the amino acid residues NLAIRLP (198-204) are predicted to be associated with sialic acid binding. The present strains have the amino acid isoleucine (I) at position 249 in the σ-1 protein, which affects the susceptibility of the type 3 σ-1 protein to cleavage by proteases in the host intestine. The present strains also have the amino acid residues 340D and 419E, which have been linked with the affinity of MRV for the central nervous system (Fig. 7).

MRuV5 and MRV were also detected in all five CPE-positive Vero cell supernatants by one-step RT-PCR, using specific primers targeting the NP, F, M, and P genes of MRuV5 and the L1 and L3 genes of MRV. The PCR products were confirmed by nucleotide sequencing using the Sanger method. The sequences of the RT-PCR products were identical to the NGS sequences of MRuV5 and MRV. The uninfected cell culture supernatants were negative for MRuV5 and MRV by RT-PCR.

Discussion

Recently, pigs have been recognised as important reservoir hosts for numerous emerging viruses that can cross species barriers to infect multiple mammalian species, including humans [42, 43]. In this study, we co-isolated MRuV5 and MRV3 from domestic pigs incidentally during a surveillance study for PEDV and TGEV, but none of the samples in the present study were positive for PEDV or TGEV. MRuV5 and MRV3 were characterised by sequencing their complete genomes. MRuV5 and MRV3 were both found to be cytopathogenic in Vero cells, causing CPE by 48 hpi. However, their individual cytopathogenicity could not be assessed due to the presence of both viruses in same samples. Similarly, the haemagglutination activity of MRuV5- and MRV3-containing Vero cell supernatants with swine RBCs could not be evaluated individually. Previous reports have shown isolation of MRuV5 strains from the respiratory system of several mammalian species [6, 8, 9, 44], including a human patient with multiple sclerosis [45]. However, there have been only a few reports of MRuV5 isolation from intestinal tissues or faecal samples [10]. Isolation of a cytopathogenic MRuV5 strain (SER) from a pig concurrently infected with porcine reproductive and respiratory syndrome virus in Germany has been reported [46]. The MRuV5 strains IND/MZ/3013789 and IND/MZ/3013814 isolated in the present study showed a high level of sequence similarity to several MRuV5 strains from China and South Korea. They did not have changes in the predicted receptor-binding site of the HN protein, the cleavage site, or the HN stalk region. The host-specificity-related amino acid residues I22, A49, R57, T254, N318, K460, and M536 observed in the HN protein of the Indian isolates differed from those (L22, S49, G57, A254, S318, T460, and T536) of MRuV5 strains from humans [47]. Similarly, host specificity variations were also observed in the fusion protein, which had the amino acid residues T3, S19, T438, L498, S530, and R536 instead of I3, G19, S438, F498, Q530, and Q536, which were reported in human strains of MRuV5 [7, 47]. Thus, the results show that the present MRuV5 strains of porcine origin are different from MRuV5 strains of human origin.

MRV strains have previously been isolated from cases of enteric infections and diarrhoea in pigs [28, 29, 31]. Moreover, MRV3 strains have been described to be mildly pathogenic to piglets, without causing diarrhoea and vomiting [31]. They have also been associated with respiratory signs in pigs, indicating their pathogenic potential at extraintestinal sites [28, 31].

The establishment of a reovirus infection in the intestine depends on proteolysis of outer-capsid proteins to yield

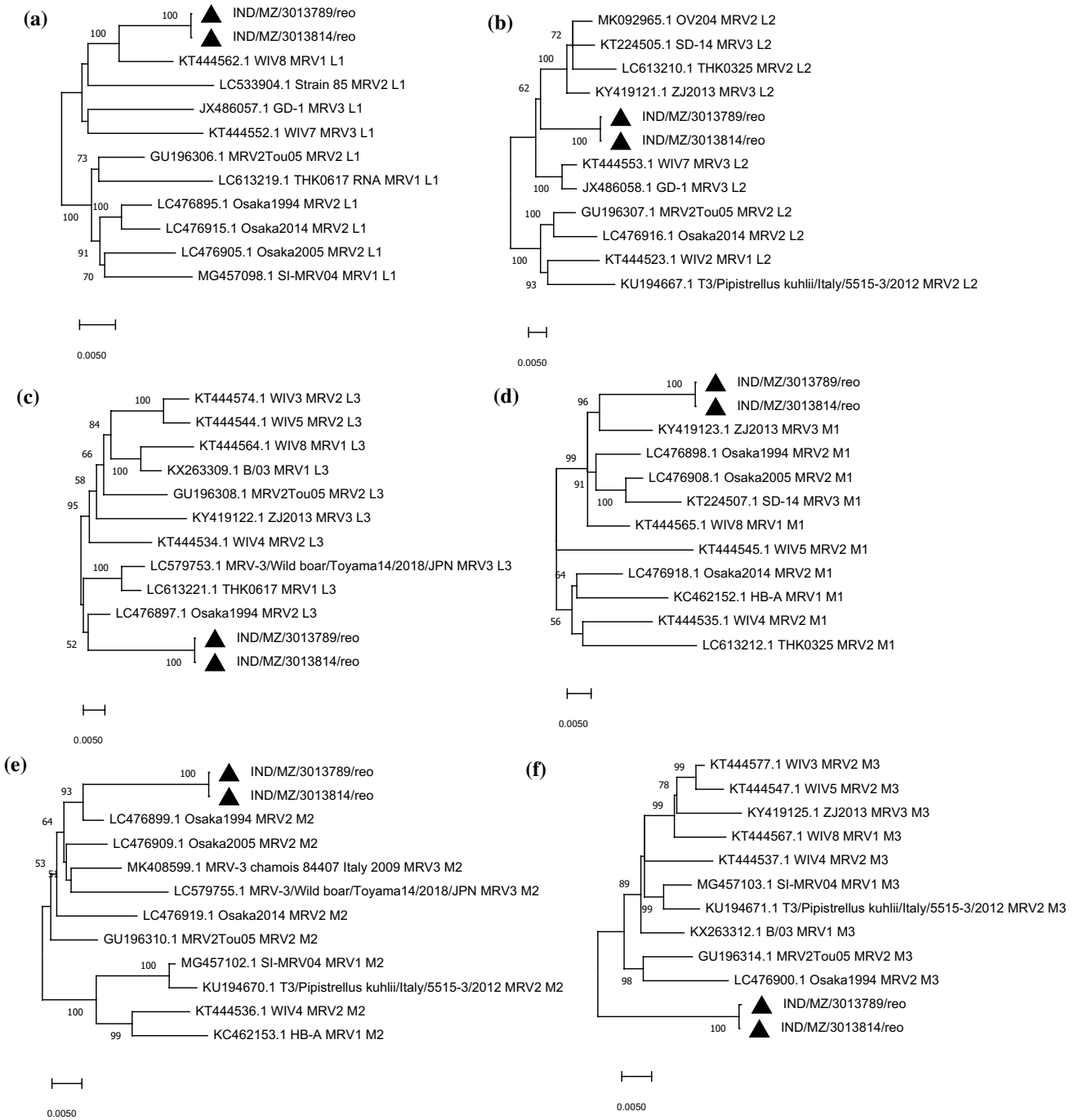

Fig. 6 Phylogenetic analysis based on different genes of MRV3. a, L1; b, L2; c, L3; d, M1; e, M2; f, M3; g, S2; h, S3; i, S4. The phylogenetic trees were constructed using MEGA X software, by the Construct/Test maximum-likelihood method, using the Tamura-Nei model and 1000 bootstrap replications. The dark triangles indicate isolates from the present study.

infectious subviral particles (ISVPs). The amino acid residue threonine at position 249 determines the susceptibility of the σ-1 protein to proteolytic cleavage [48]. The presence of isoleucine (I) at position 249 in the σ-1 protein of the Indian MRV3 isolates indicates its resistance to cleavage by trypsin. The amino acid sequence NLAIRLP (positions 198-204), which are part of the sialic acid binding site, was present in both MRV3 isolates from the present study [49]. Moreover, two amino acids residues (340D and 419E) present in the σ-1 protein of MRV3 in the present study have been reported to be associated with the neurotropism of orthoreoviruses [22]. MRV type 3 strains with protease-resistant σ-1 protein

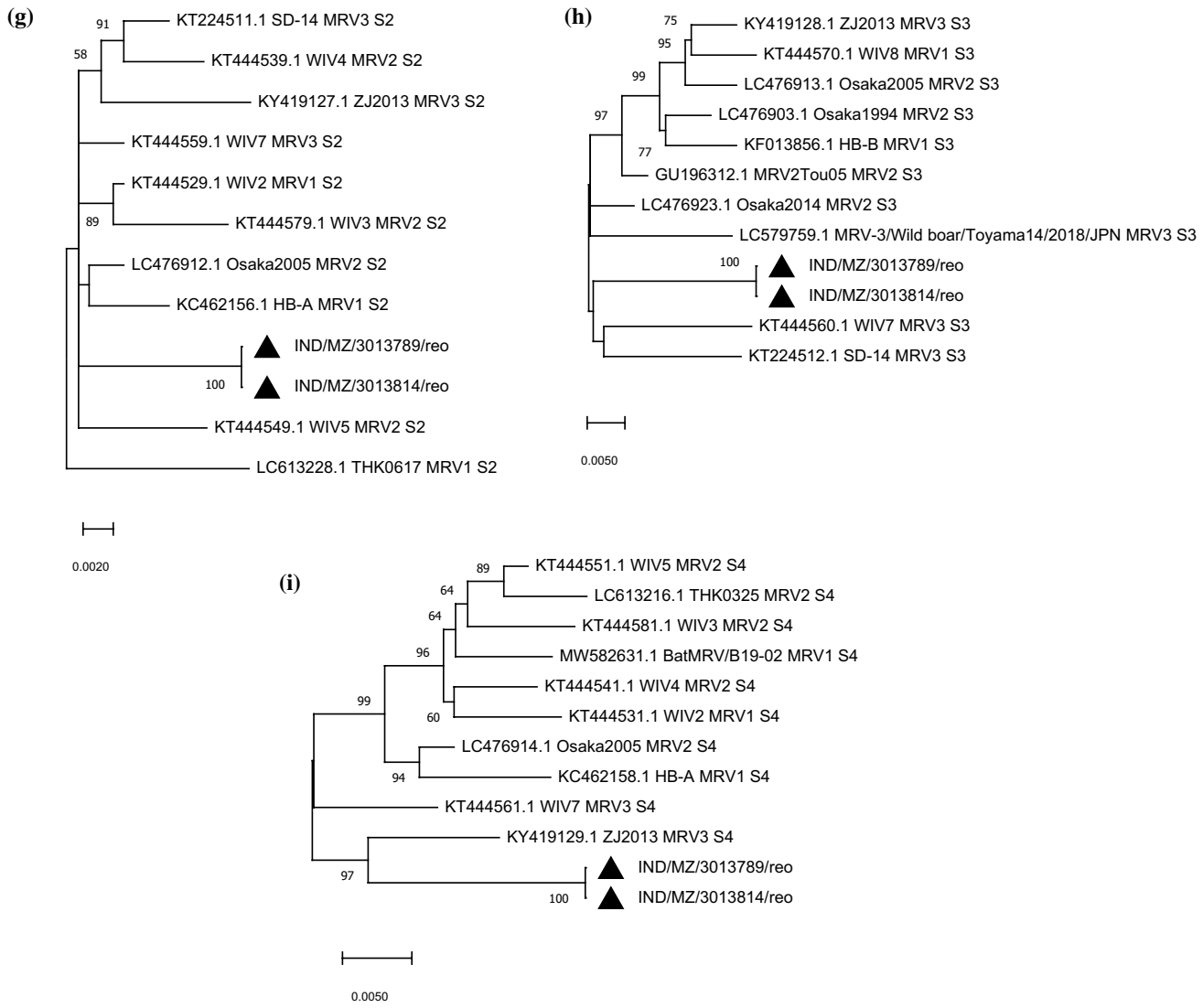

Fig. 6 (continued)

are able to migrate and replicate in other organ systems, including the central nervous system [48]. Thus, the presence of isoleucine (I) instead of threonine at position 249, along with 340D and 419E in the σ-1 protein, indicates that the Indian MRV strains may have potentially neurotropic features similar to those of other strains reported earlier [49, 50].

Phylogenetically, the Indian MRV3 strains in the present study clustered in lineage IV [24]. All of the segments of MRV3 strains IND/MZ/3013789/reo and IND/MZ/3013814/reo have the highest nucleotide sequence similarity to isolates from pigs and bats in China, pigs in South Korea, and humans in Japan, indicating the circulation of MRVs with common genetic signature in Asian countries. The nucleotide sequences of all of the segments except S1 showed similarity to the corresponding sequences of each of the three MRV serotypes, indicating that these segments are more conserved in MRVs across serotypes. Reoviruses have a segmented genome that can undergo genetic reassortment due to intra- and interspecies transmission, resulting in increased genetic diversity [14, 51]. Reassortment of genome segments among different MRV serotypes play an important role in the emergence of new variants of MRV, and reassortment between MRV strains have been reported earlier [13, 14, 30, 31].

This is the first report of co-isolation and whole-genomic characterisation of MRuV5 and MRV3 from domestic pigs in India, confirming the role of pigs as animal reservoir

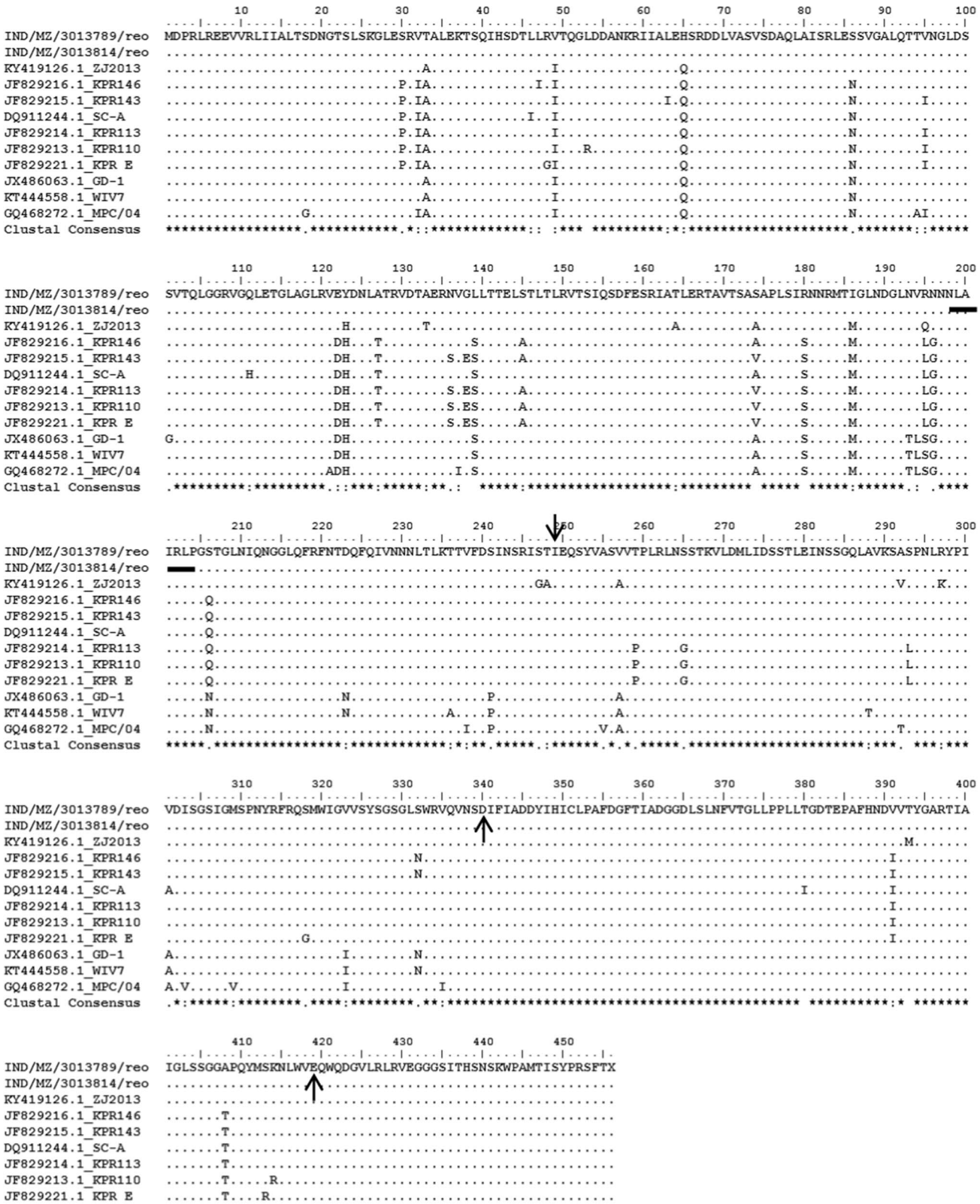

Fig. 7 Alignment and comparison of the deduced amino acid sequences of the σ-1 proteins of the IND/MZ/3013789/reo and IND/MZ/3013814/reo strains of MRV3 with those of Asian MRV3 strains. Amino acids predicted to be part of the sialic acid-binding site are underlined, amino acid involved in resistance to cleavage by intestinal proteases is indicated by a downward arrow, and the amino acids predicted to be involved in neurotropism are indicated by upward arrows.

hosts. However, the origin and prevalence of these viruses and their pathogenic role in swine were not elucidated. Nevertheless, mild subclinical symptoms or sporadic severe infections caused by these viruses cannot be ruled out, because comprehensive data were not available for the backyard pigs. The observation of faecal shedding of MRuV5 and MRV3 indicates the likely role of environmental contamination in the spread of these viruses, possibly through fomites. In addition, close human-animal interactions can promote the spread of emerging viruses [2, 33]. Since there are no species barriers in the transmission of MRuV5 and MRV3, they may spread from animals to humans and vice versa, facilitating the emergence of novel virus strains across different geographical areas.

In conclusion, new information about the circulation of MRuV5 and MRV3 in pigs in India has been gained for the first time. The results of the present study are of interest for systematic studies of the epidemiology, genetic evolution, host adaptability, and pathogenic potential of these viruses in mammalian reservoirs and their possible spread or transmission to domestic animals and humans.

Acknowledgements The authors are thankful to the Indian Council of Agricultural Research, New Delhi, for funding to carry out this study. We also thank the staff of the Department of Animal Husbandry and Veterinary of the Government of Mizoram, Assam and Chhattisgarh, who helped in collection of blood and faecal samples from pigs.

Author contributions All authors contributed to the study conception and design. Samples were collected by FS, KR, DS, and GV. Material preparation, laboratory tests and analyses were performed by FS, KR, DS, DS, SK, and SKJ. The first draft of the manuscript was written by FS and KR, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The present research work was carried out under the institutional project entitled "Diagnostic preparedness for porcine epidemic diarrhoea and transmissible gastroenteritis in pigs" (Project code IXX 13779) funded by the Indian Council of Agricultural Research, New Delhi, through institutional research contingency.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Research involving human participants and/or animals This study did not involve any human participants or animals.

References

1. Woolhouse M, Scott F, Hudson Z, Howey R, Chase-Topping M (2012) Human viruses: discovery and emergence. Philos Trans R Soc Lond B Biol Sci 367:2864–2871. https://doi.org/10.1098/rstb.2011.0354
2. Parvez MK, Parveen S (2017) Evolution and emergence of pathogenic viruses: past, present, and future. Intervirology 60:1–7. https://doi.org/10.1159/000478729
3. Chan JFW, To KKW, Tse H, Jin DY, Yuen KY (2013) Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol 21:544–555. https://doi.org/10.1016/j.tim.2013.05.005
4. Terrier O, Rolland JP, Rosa-Calatrava M, Lina B, Thomas D, Moules V (2009) Parainfluenza virus type 5 (PIV-5) morphology revealed by cryo-electron microscopy. Virus Res 142:200–203. : https://doi.org/10.1016/j.virusres.2008.12.017
5. Henrickson KJ (2003) Parainfluenza viruses. Clin Microbiol Rev 16:242–264. https://doi.org/10.1128/CMR.16.2.242-264.2003
6. Lee YN, Lee C (2013) Complete genome sequence of a novel porcine parainfluenza virus 5 isolate in Korea. Ach Virol 158:1765–1772. https://doi.org/10.1007/s00705-013-1770-z
7. Chatziandreou N, Stock N, Young D, Andrejeva J, Hagmaier K, McGeoch DJ, Randall RE (2004) Relationships and host range of human, canine, simian and porcine isolates of simian virus 5 (parainfluenza virus 5). J Gen Virol 85:3007–3016. https://doi.org/10.1099/vir.0.80200-0
8. Liu Y, Li N, Zhang S, Zhang F, Lian H, Hu R (2015) Parainfluenza virus 5 as possible cause of severe respiratory disease in calves, China. Emerg Infect Dis 21:2242–2244. https://doi.org/10.3201/eid2112.141111
9. Zhai JQ, Zhai SL, Lin T, Liu JK, Wang HX, Li B, Zhang H, Zou SZ, Zhou X, Wu MF, Chen W, Luo ML (2017) First complete genome sequence of parainfluenza virus 5 isolated from lesser panda. Arch Virol 162:1413–1418. https://doi.org/10.1007/s00705-017-3245-0
10. Jiang N, Wang E, Guo D, Wang X, Su M, Kong F, Yuan D, Zhai J, Sun D (2018) Isolation and molecular characterization of parainfluenza virus 5 in diarrhea-affected piglets in China. J Vet Med Sci 80:590–593. https://doi.org/10.1292/jvms.17-0581
11. Shatkin AJ, Sipe JD, Loh P (1968) Separation of ten reovirus genome segments by polyacrylamide gel electrophoresis. J Virol 2:986–991. https://doi.org/10.1128/JVI.2.10.986-991.1968
12. Nibert ML, Schiff LA (2001) Reoviruses and their replication. In: Knipe DM, Howley PM (eds) Fields Virology, 4th edn. Lippincott Williams and Wilkins, Philadelphia, pp 1679–1728
13. Lelli D, Moreno A, Steyer A, Nagli˘c T, Chiapponi C, Prosperi A, Faccin F, Sozzi E, Lavazza A (2015) Detection and characterization of a novel reassortant mammalian orthoreovirus in bats in Europe. Viruses 7:5844–5854. https://doi.org/10.3390/v7112908
14. Rosa UA, Ribeiro GO, Villanova F, Luchs A, Milagres FAP, Komninakis SV, Tahmasebi R, Lobato MCABS, Brustulin R, Chagas RTD, Abrão MFNDS, Soares CVDA, Tinker RJ, Pandey RP, Raj VS, Sabino EC, Deng X, Delwart E, Costa ACD, Leal É (2019) First identification of mammalian orthoreovirus type 3 by gut virome analysis in diarrheic child in Brazil. Sci Rep 9:18599. https://doi.org/10.1038/s41598-019-55216-5
15. Fields BN (1982) Molecular basis of Reovirus virulence. Arch Virol 71:95–107. https://doi.org/10.1007/BF01314880
16. Furlong DB, Nibert ML, Fields BN (1988) Sigma 1 protein of mammalian reoviruses extends from the surfaces of viral particles. J Virol 62:246–256. https://doi.org/10.1128/JVI.62.1.246-256.1988
17. Fraser RD, Furlong DB, Trus BL, Nibert ML, Fields BN, Steven AC (1990) Molecular structure of the cell-attachment protein of reovirus: correlation of computer-processed electron micrographs with sequence-based predictions. J Virol 64:2990–3000. : https://doi.org/10.1128/JVI.64.6.2990-3000.1990
18. Weiner HL, Fields BN (1977) Neutralization of reovirus: the gene responsible for the neutralization antigen. J Exp Med 146:1305–1310. https://doi.org/10.1084/jem.146.5.1305

19. Weiner HL, Raming RF, Mustoe TA, Fields BN (1978) Identification of the gene coding for the hemagglutinin of reovirus. Virol 86:581–584. https://doi.org/10.1016/0042-6822(78)90099-5
20. Weiner HL, Ault KA, Fields BN (1980) Interaction of reovirus with cell surface receptors. I. Murine and human lymphocutes have a receptor for the emagglutinin of reovirus type 3. J Immunol 124:2143–2148
21. Lee PW, Hayes EC, Joklik WK (1981) Protein s1 is the reovirus cell attachment protein. Virol 108:156–163. https://doi.org/10.1016/0042-6822(81)90535-3
22. Bassel-Duby R, Spriggs DR, Tyler KL, Fields BN (1986) Identification of attenuating mutations on the reovirus type 3 S1 double-stranded RNA segment with a rapid sequencing technique. J Virol 60:64–67. https://doi.org/10.1128/JVI.60.1.64-67.1986
23. Day JM (2009) The diversity of the orthoreoviruses: molecular taxonomy and phylogentic divides. Infect Genet Evol 9:390–400. https://doi.org/10.1016/j.meegid.2009.01.011
24. Kwon HJ, Kim HH, Kim HJ, Park JG, Son KY, Jung J, Lee WS, Cho KO, Park SJ, Kang MI (2012) Detection and molecular characterization of porcine type 3 orthoreoviruses circulating in South Korea. Vet Microbiol 157:456–463. https://doi.org/10.1016/j.vetmic.2011.12.032
25. Tyler KL (2001) Mammalian reoviruses. In: Knipe DM, Howley PM (Eds), Fields Virology. 4thEd. Lippincott Williams and Wilkins, Philadelphia, pp 1729–45
26. Tyler KL, Barton ES, Ibach ML, Robinson C, Campbell JA, O'Donnell SM, Valyi-Nagy T, Clarke P, Wetzel JD, Dermody TS (2004) Isolation and molecular characterization of a novel type 3 reovirus from a child with meningitis. J Infect Dis 189:1664–1675. https://doi.org/10.1086/383129
27. Steyer A, Gutiérrez-Aguire I, Kolenc M, Koren S, Kutnjak D, Pokorn M, Poljšak-Prijatelj M, Racki N, Ravnikar M, Sagadin M, Fratnik Steyer A, Toplak N (2013) High similarity of novel orthoreovirus detected in a child hospitalized with acute gastroenteritis to mammalian orthoreoviruses found in bats in Europe. J Clin Microbiol 51:3818–3825. https://doi.org/10.1128/JCM.01531-13
28. Zhang C, Liu L, Wang P, Liu S, Lin W, Hu F, Wu W, Chen W, Cui S (2011) A potentially novel reovirus isolated from swine in northeastern China in 2007. Virus Genes 43:342–349. https://doi.org/10.1007/s11262-011-0642-4
29. Thimmasandra Narayanappa A, Sooryanarain H, Deventhiran J, Cao D, Ammayappan Venkatachalam B, Kambiranda DM, LeRoith T, Heffron CL, Lindstrom NM, Hall K, Jobst PM, Sexton C, Meng X, Elankumaran S (2015) A novel pathogenic mammalian orthoreovirus from diarrheic pigs and swine blood meal in the United States. MBio 6:593. https://doi.org/10.1128/mBio.00593-15
30. Lelli D, Beato MS, Cavicchio L, Lavazza A, Chiapponi C, Leopardi S, Baioni L, Benedictis PD, Moreno A (2016) First identification of mammalian orthoreovirus Type 3 in diarrheic pigs in Europe. Virol J 13:139. https://doi.org/10.1186/s12985-016-0593-4
31. Qin P, Li H, Wang JW, Wang B, Xie RH, Xu H, Zhao LY, Li L, Pan Y, Song Y, Huang YW (2017) Genetic and pathogenic characterization of a novel reassortant mammalian orthoreovirus 3 (MRV3) from a diarrheic piglet and seroepidemiological survey of MRV3 in diarrheic pigs from east China. Vet Microbiol 208:126–136. https://doi.org/10.1016/j.vetmic.2017.07.021
32. Dikid T, Jain SK, Sharma A, Kumar A, Narain JP (2013) Emerging & re-emerging infections in India: an overview. Indian J Med Res 138:19–31
33. Borkenhagen LK, Mallinson KA, Tsao RW, Ha SJ, Lim WH, Toh TH, Anderson BD, Fieldhouse JK, Philo SE, Chong KS, Lindsley WG, Ramirez A, Lowe JF, Coleman KK, Gray GC (2018) Surveillance for respiratory and diarrheal pathogens at the human-pig interface in Sarawak, Malaysia. PLoS ONE 13:e0201295. https://doi.org/10.1371/journal.pone.0201295
34. Kim O, Choi C, Kim B, Chae C (2000) Detection and differentiation of porcine epidemic diarrhoea virus and transmissible gastroenteritis virus in clinical samples by multiplex RT-PCR. Vet Rec 146:637–640. https://doi.org/10.1128/jcm.30.6.1365-1373.1992
35. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK (1992) Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol 30:1365–1373. https://doi.org/10.1128/jcm.30.6.1365-1373.1992
36. Boros A, Pankovics P, Reuter G (2011) Characterization of a novel porcine enterovirus in domestic pig in Hungary. Infect Genet Evol 11:1096–1102. https://doi.org/10.1016/j.meegid.2011.04.003
37. Krumbholz A, Wurm R, Scheck O, Birch-Hirschfeld E, Egerer R, Henke A, Wutzler P, Zell R (2003) Detection of porcine teschoviruses and enteroviruses by LightCycler real-time PCR. J Virol Methods 113:51–63. https://doi.org/10.1016/s0166-0934(03)00227-1
38. Liu JK, Wei CH, Yang XY, Dai AL, Li XH (2015) Simultaneous detection and differentiation of porcine circovirus type 2, type 2 porcine reproductive and respiratory syndrome virus, porcine parvovirus and pseudorabies virus in pigs with postweaning multisystemic wasting syndrome (PMWS) by multiplex PCR. Vet Arh 85:511–521
39. Decaro N, Campolo M, Desario C, Ricci D, Camero M, Lorusso E, Elia G, Lavazza A, Martella V, Buonavoglia C (2005) Virological and molecular characterization of a mammalian orthoreovirus type 3 strain isolated from a dog in Italy. Vet Microbiol 109:19–27. https://doi.org/10.1016/j.vetmic.2005.05.014
40. Leary TP, Erker JC, Chalmers ML, Cruz AT, Wetzel JD, Desai SM, Mushahwar IK, Dermody TS (2002) Detection of mammalian reovirus RNA by using reverse transcription-PCR: sequence diversity within the lambda3-encoding L1 gene. J Clin Microbiol 40:1368–1375. https://doi.org/10.1128/JCM.40.4.1368-1375.2002
41. Ouattara LA, Barin F, Barthez MA, Bonnaud B, Roingeard P, Goudeau A, Castelnau P, Vernet G, Paranhos-Baccalà G, Komurian-Pradel F (2011) Novel human reovirus isolated from children with acute necrotizing encephalopathy. Emerg Infect Dis 17:1436–1444. https://doi.org/10.3201/eid1708.101528
42. Meng XJ (2012) Emerging and re-emerging swine viruses. Transbound Emerg Dis 59:85–102. https://doi.org/10.1111/j.1865-1682.2011.01291.x
43. Perfumo CJ, Pereda A, Jongkaewwattana A, Chen Z, Perez DR, Ma J (2020) Editorial: emerging swine viruses. Front Vet Sci 7:132. https://doi.org/10.3389/fvets.2020.00132
44. Liu C, Li X, Zhang J, Yang L, Li F, Deng J, Tan F, Sun M, Liu Y, Tian K (2017) Isolation and genomic characterization of a canine parainfluenza virus type 5 strain in China. Arch Virol 162:2337–2344. https://doi.org/10.1007/s00705-017-3387-0
45. Goswami KK, Lange LS, Mitchell DN, Cameron KR, Russell WC (1984) Does simian virus 5 infect humans? J Gen Virol 65:1295–1303. https://doi.org/10.1099/0022-1317-65-8-1295
46. Heinen E, Herbst W, Schmeer N (1998) Isolation of a cytopathogenic virus from a case of porcine reproductive and respiratory syndrome (PRRS) and its characterization as parainfluenza virus 2. Arch Virol 143:2233–2239. https://doi.org/10.1007/s007050050454

47. Charoenkul K, Nasamran C, Janetanakit T, Chaiyawong S, Bunpapong N, Boonyapisitsopa S, Tangwangvivat R, Amonsin A (2021) Molecular detection and whole genome characterization of Canine Parainfluenza type 5 in Thailand. Sci Rep 11:3866. https://doi.org/10.1038/s41598-021-83323-9
48. Chappell JD, Barton ES, Smith TH, Baer GS, Duong DT, Nibert ML, Dermody TS (1998) Cleavage susceptibility of reovirus attachment protein sigma1 during proteolytic disassembly of virions is determined by a sequence polymorphism in the sigma1 neck. J Virol 72:8205–8213. https://doi.org/10.1128/JVI.72.10.8205-8213.1998
49. Lelli D, Moreno A, Lavazza A, Bresaola M, Canelli E, Boniotti MB, Cordioli P (2013) Identification of Mammalian orthoreovirus type 3 in Italian bats. Zoonoses Public Health 60:84–92. https://doi.org/10.1111/zph.12001
50. Besozzi M, Lauzi S, Lelli D, Lavazza A, Chiapponi C, Pisoni G, Viganò R, Lanfranchi P, Luzzago C (2019) Host range of mammalian orthoreovirus type 3 widening to alpine chamois. Vet Microbiol 230:72–77. https://doi.org/10.1016/j.vetmic.2019.01.012
51. McDonald SM, Nelson MI, Turner PE, Patton JT (2016) Reassortment in segmented RNA viruses: mechanisms and outcomes. Nat Rev Microbiol 14:448–460. https://doi.org/10.1038/nrmicro.2016.46