ORIGINAL ARTICLE

Veterinary Research Forum. 2025; 16 (10): 585 - 590
doi: 10.30466/vrf.2025.2039338.4410
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Molecular detection and phylogenetic analysis of feline panleukopenia virus in domestic cat population of Mizoram state, India

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Article Info

Article history:

Received: 22 August 2024
Accepted: 18 February 2025
Available online: 15 October 2025

Keywords:

FPV
Mizoram
Molecular surveillance
Pathology
Polymerase chain reaction

Abstract

The most significant infectious disease that affects cats is thought to be feline panleukopenia, also known as Cat distemper. Despite its epidemiological status, few literatures are available regarding the clinic-pathological aspect of the disease and about the molecular epidemiology of the circulating feline panleukopenia virus (FPV) in India. This study gives a comprehensive insight into the prevalence, pathology and diagnosis of FPV in cat population of Mizoram. Twenty-six cats that died of clinical disease suspected of FPV were subjected to a thorough pathological examination followed by molecular diagnosis. The FPV infection was confirmed in 12 out of the 26 cats by polymerase chain reaction assay targeting the *VP2* gene of FPV. The phylogenetic analysis based on the full *VP2* gene of FPV has demonstrated close genetic affinity of FPV strains circulating in Mizoram with the isolates from Thailand (MW589472), Italy (MZ508524) and China (OR727315). The analysis of the VP2-deduced amino acid sequence revealed two distinct mutations, S179T and I401V, exclusively identified in isolates from this particular study.

Introduction

Feline Panleukopenia is a highly contagious disease caused by feline panleukopenia virus (FPV), which causes severe illness in cats, particularly in kittens of less than 1 year of age with a high mortality rate of up to 90.00%.[1,2] It affects all the members of the Felidae, as well as minks, raccoons and foxes and is also known as cat distemper and feline plague.[3] Feline panleukopenia is caused by *Canine protoparvovirus-1* which belongs to the family *Parvoviridae* and subfamily *Parvovirinae*. It is a small, non-enveloped linear single-stranded DNA virus with a genome size of 5.10 kb consisting of two major genes i.e., the nonstructural (NS) protein gene and the structural protein gene. The NS gene encodes the *NS1* and *NS2* proteins involved in DNA replication, capsid assembly and intracellular transport. The structural gene encodes capsid virus proteins *VP1* and *VP2*. The viral capsid is comprised of 60 protein subunit molecules arranged in icosahedral symmetry.[4]

Feline panleukopenia virus may result in clinical disease ranging from subclinical infection to peracute syndrome with sudden death. The disease is characterized by severe depression, high fever, lethargy and anorexia. Affected cats suffer from vomiting and develop watery to hemorrhagic diarrhea.[5,6] In recent years, cats have become a trendy pet for the urban population in India. The pet cat population in India is estimated to be nearly 3.60 million in the year 2023. Even though FPV is one of the most serious illnesses, information regarding the prevalence, pathology and molecular epidemiology from India is lacking. In the present study we studied the pathology of field cases of FPV in domestic cat population of Mizoram, India confirmed the cases by detection of *VP2* gene of FPV and characterized the circulating strain based on the full *VP2* gene of FPV.

Materials and Methods

Samples and epidemiological data for the current study were collected for the period from March 2022 to August 2023. Different private clinics and government veterinary hospitals located in the Aizawl district of Mizoram were regularly visited and feline cases with ailments were monitored. Dead feline carcasses suspected of FPV were collected with detailed clinical history(including sex, age, breed, castrated/spayed, duration of the clinical signs, and symptoms) and a thorough post-mortem examination was

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performed. Gross lesions were recorded and representative tissue samples comprising different parts of the intestine, stomach, liver, mesenteric lymph nodes, kidneys, lungs, heart, brain and bone marrow were collected. For histopathological procedures, representative tissues were collected and preserved in 10.00% neutral buffered formalin and also preserved at –80.00 ˚C for molecular diagnosis. Formalin-fixed tissues were processed and stained with routine Hematoxylin and Eosin stain.[7]

Total DNA from the tissue samples was extracted using Phenol Chloroform Isoamyl method,[8] and subjected to conventional polymerase chain reaction assay for detection of the *VP2* gene of FPV.[9] The amplified products were viewed in 1.50% agarose gel electrophoresis and the size was compared to a 100 bp DNA ladder (GeneRuler, SM0243; Thermo Scientific, Waltham, USA). Further, the full *VP2* gene of FPV was amplified using published primers.[10] The amplified product was purified using the GeneJet Gel Extraction Kit (K0691, Thermo Scientific), cloned in PTZ57R/T vector and sequenced at DNA sequencing facility, Delhi University, South Campus, New Delhi. The generated sequences were analyzed using Mega Software (version X; Biodesign Institute, Tempe, USA),[11] and submitted to GenBank®, NCBI, and the accession numbers were obtained (accession no PP035815, PP035816 and PP035817).

For phylogenetic analysis, a total number of 85 reference *VP2* full gene sequences were retrieved from GenBank®. The reference sequences were selected to include FPV sequences from different countries from different years along with the metadata such as host, date and place of collection (Table 1). The collected reference sequences along with the sequences from this study were aligned and deduced to get amino acid sequences by using MEGA X.[11] To detect any recombinant, recombination analysis was performed using RDP4 software (version 4.101; University of Cape Town, Cape Town, South Africa).[12] Algorithms such as Chimaera, Bootstcan, RDP4, GENECONV and Maxchi were utilized to detect the recombinants with a p-value < 0.05. A p-value of < 0.05 was used for statistical significance. Bayesian inference method was used for performing phylogenetic analysis utilizing BEAST package (version 2.7; University of Auckland, Auckland, New Zealand).[13] Tracer software (version 1.7.2; University of Auckland) was used for analyzing the BEAST result files.[14] FigTree software (version 1.4.4; University of Edinburgh, Edinburgh, UK) was used for visualizing the tree file. Nucleotide substitution model selection was done by Mega X software and Hasegawa–Kishino–Yano model + Gamma distribution + Invariant sites (HKY + G + I) was chosen as the best-fit model. Relaxed clock log normal and Coalescent Bayesian Skyline models were used as clock model and tree prior, respectively. Markov chain Monte Carlo chain length of 100 million were used to ensure adequate effective sample size. The obtained trees file was summarized using tree annotator software to get Maximum clade credibility tree which was visualized in FigTree.

Results

Twenty-six cats that died after clinical disease suspected for FPV during the period from March 2022 to August 2023 were subjected to a detailed post-mortem examination with consent from the owners. The gross lesions were recorded and representative tissue samples were collected from all the cases for histopathological and molecular diagnosis. Twelve of the cases were tested to be positive for FPV infection.

All the affected cats were mixed-breed cats from the local area. Eight of them were vaccinated against FPV (Feligen®CRP/L; Virbac, Carros, France) while four of them were not vaccinated. The most severe clinical disease was observed in the cats of less than one year of age (8/12) compared to that of the cats of above one year of age group (4/12). Affected cats showed severe depression and suffered from high fever (40.00 to 42.22 ˚C), anorexia, vomiting, diarrhea and severe dehydration. The detailed post-mortem examination of affected cats that died of FPV showed hemorrhagic gastroenteritis, pneumonia, nephritis and lymphadenopathy. Petechial or ecchymotic hemorrhages on the serosal surface of the intestine were observed frequently. The jejunum and ileum were the most severely affected part of the intestine and were distended with foul-smelling, blood and mucus mixed with watery intestinal content (Fig. 1A). Lungs were congested with focal areas of hemorrhages. Kidneys were swollen with white spots on the cortical surfaces. Spleen and mesenteric lymph nodes were severely congested and hemorrhagic.

Microscopical examination of the intestine revealed dilated and distended intestinal crypts filled with mucus and desquamated necrotic cell debris. Necrosis and desquamation of crypt epithelium in many places led to the formation of cysts or empty spaces leaving only the basement membrane (Fig. 1B). Lungs showed areas of emphysema and severe congestion, hemorrhages in the alveolar wall with infiltration of mononuclear cells. Coagulative necrosis of tubular epithelium, infiltration of mononuclear inflammatory cells in the interstitium and severe congestion in the glomeruli were observed in the kidney (Fig. 1C). The spleen lesion displayed a regenerative white pulp with widespread hemorrhages in the red pulp (Fig. 1D). Polymerase chain reaction assay targeting the full VP2 sequence of FPV (1,755 bp) yielded the desired amplification and confirmed the FPV infection in twelve out of the total 26 cats studied.

The phylogenetic analysis based on complete *VP2* gene sequences of FPV was performed to understand the evolutionary relationship of circulating FPV in the cat population of Mizoram. The analysis confirmed a consistent genetic evolutionary closeness among the three FPV isolates. The phylogenetic tree was constructed using Bayesian inference methods (Fig. 2). All three isolates from this study were grouped with sequences from Italy (EU498682) and Hungary (EU360959). The phylogenetic clustering revealed no significant relation with the time of sample collection or geographical location. The time to the most recent common ancestor (tMRCA) for the dataset was estimated to be 1,888.37 (95.00% highest posterior density [HPD] 1,773.29 to 1,960.28), and the mutation rate was calculated as 6.082×10^{-5} with 2.572 - 9.570 × 10^{-5}. The sequence analysis of the complete *VP2* gene of circulating FPV revealed nucleotide homology of 99.99% among themselves and maximum nucleotide similarity with FPV strains from Thailand (99.38%, MW589472 (2020), Italy (99.33%, MZ508524) and China (99.22%, OR727315).

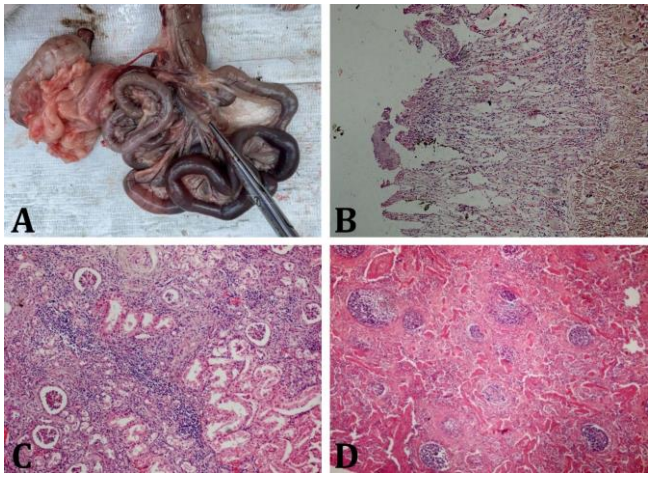

Fig. 1. A) Severely congested, hemorrhagic enteritis and mesenteric lymph node, **B)** Duodenum shows denuded villi, hemorrhages and infiltration of inflammatory cells, **C)** Kidney shows tubular coagulative necrosis, infiltration of inflammatory cells in interstitium, glomeruli with dilated Bowman's space and thickened Bowman's capsule, and **D)** Spleen shows lymphoid depletion, extremely prominent red pulp with congestion and hemorrhages (Hematoxylin and Eosin staining; B and C: 200×, D: 100×).

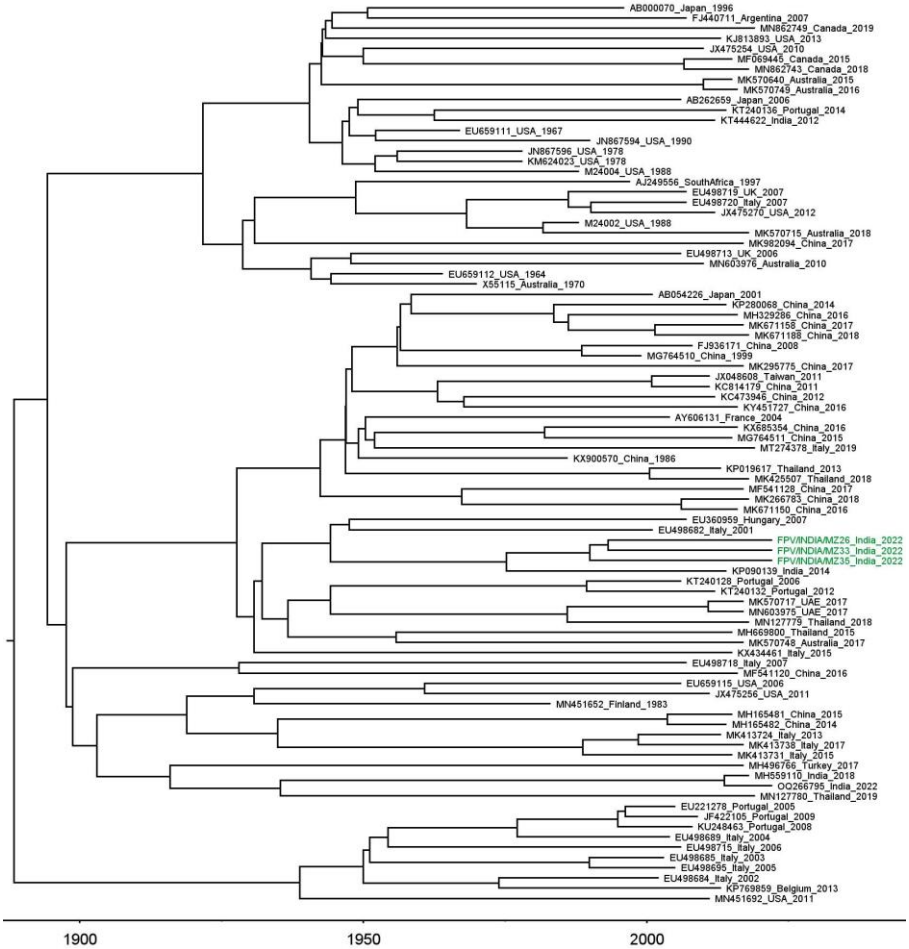

Fig. 2. Maximum clade credibility tree based on full VP2 of reconstructed using Bayesian inference methods. The taxa names are indicated with GenBank® Accession number, country name and year of sample collection. The sequences from this study are labelled with green.

Table 1. Details of *VP2* gene sequences of feline panleukopenia virus used in the analysis.

No.	Accession No.	Location	Year	No.	Accession No.	Location	Year
1	FPV/INDIA/MZ26	India	2022	**45**	KU248463	Portugal	2008
2	FPV/INDIA/MZ33	India	2022	**46**	KX434461	Italy	2015
3	FPV/INDIA/MZ35	India	2022	**47**	KX685354	China	2016
4	AB000070	Japan	1996	**48**	KX900570	China	1986
5	AB054226	Japan	2001	**49**	KY451727	China	2016
6	AB262659	Japan	2006	**50**	M24002	USA	1988
7	AJ249556	South Africa	1997	**51**	M24004	USA	1988
8	AY606131	France	2004	**52**	MF069445	Canada	2015
9	EU221278	Portugal	2005	**53**	MF541120	China	2016
10	EU360959	Hungary	2007	**54**	MF541128	China	2017
11	EU498682	Italy	2001	**55**	MG764510	China	1999
12	EU498684	Italy	2002	**56**	MG764511	China	2015
13	EU498685	Italy	2003	**57**	MH165481	China	2015
14	EU498689	Italy	2004	**58**	MH165482	China	2014
15	EU498695	Italy	2005	**59**	MH329286	China	2016
16	EU498713	UK	2006	**60**	MH496766	Turkey	2017
17	EU498715	Italy	2006	**61**	MH559110	India	2018
18	EU498718	Italy	2007	**62**	MH669800	Thailand	2015
19	EU498719	UK	2007	**63**	MK266783	China	2018
20	EU498720	Italy	2007	**64**	MK295775	China	2017
21	EU659111	USA	1967	**65**	MK413724	Italy	2013
22	EU659112	USA	1964	**66**	MK413731	Italy	2015
23	EU659115	USA	2006	**67**	MK413738	Italy	2017
24	FJ440711	Argentina	2007	**68**	MK425507	Thailand	2018
25	FJ936171	China	2008	**69**	MK570640	Australia	2015
26	JF422105	Portugal	2009	**70**	MK570715	Australia	2018
27	JN867594	USA	1990	**71**	MK570717	UAE	2017
28	JN867596	USA	1978	**72**	MK570748	Australia	2017
29	JX048608	Taiwan	2011	**73**	MK570749	Australia	2016
30	JX475254	USA	2010	**74**	MK671150	China	2016
31	JX475256	USA	2011	**75**	MK671158	China	2017
32	JX475270	USA	2012	**76**	MK671188	China	2018
33	KC473946	China	2012	**77**	MK982094	China	2017
34	KC814179	China	2011	**78**	MN127779	Thailand	2018
35	KJ813893	USA	2013	**79**	MN127780	Thailand	2019
36	KM624023	USA	1978	**80**	MN451652	Finland	1983
37	KP019617	Thailand	2013	**81**	MN451692	USA	2011
38	KP090139	India	2014	**82**	MN603975	UAE	2017
39	KP280068	China	2014	**83**	MN603976	Australia	2010
40	KP769859	Belgium	2013	**84**	MN862743	Canada	2018
41	KT240128	Portugal	2006	**85**	MN862749	Canada	2019
42	KT240132	Portugal	2012	**86**	MT274378	Italy	2019
43	KT240136	Portugal	2014	**87**	OQ266795	India	2022
44	KT444622	India	2012	**88**	X55115	Australia	1970

Discussion

Feline panleukopenia virus causes fatal leukopenia and severe hemorrhagic diarrhea in cats. Although FPV have been reported, the biological and genetic features of Indian FPVs remain unclear. There is no report of FPV infection in cats from North East Region, India. The present study aimed to understand the occurrence, pathology, diagnosis and molecular epidemiology of FPV infection in cat population of Mizoram state of North East India. The study identified FPV as a major cause of mortality in cat population of Mizoram. Out of the total twenty-six domestic cat that died of gastroenteritis, twelve were confirmed as died of FPV by pathological examination followed by detection of the *VP2* gene of FPV in tissue lesions by polymerase chain reaction.

The tropism of FPV is restricted to highly dividing cells such as those found in the intestine, bone marrow or lymphoid tissues as the parvovirus replication takes place in the nucleus and requires cells in synthesis phase.[15] This study on naturally occurring FPV infection also recorded the most prominent pathological lesion in intestine (jejunum and ileum) followed by mesenteric lymph nodes, spleen, lungs and kidneys. The lesions caused by FPV

infection in intestine and lymphoid organs are well-established.[16] However, additionally we also recorded severe interstitial nephritis with tubular degenerative changes in the affected cats.

The evolutionary relationship of circulating FPV in the cat population of Mizoram, exhibited a close genetic affinity to isolates from Thailand (MW589472), Italy (MZ508524) and China (OR727315). The tMRCA for the dataset was estimated to be 1,888.37 (95.00% HPD 1,773.29 to 1,960.28), and the mutation rate was calculated as 6.082×10^{-5} with $2.572 - 9.570 \times 10^{-5}$. This was slightly higher (2.35×10^{-4}) than earlier estimation at 1920s (95.00% HPD 1,910.48 - 1,934.99) and 6.082×10^{-5} with $2.572 - 9.570 \times 10^{-5}$, respectively.[17] These disparities in estimated tMRCA and mutation rates suggested potential variations in evolutionary timelines and rates among FPV strains from different geographical regions. Understanding these differences could shed light on diverse viral characteristics and transmission patterns.

The VP2 capsid protein of FPV contains the major antigenic determinant. It plays an important role in detecting viral pathogenicity, and amino acid changes in the capsid protein are important molecular determinants in the host range. The VP2 protein can self-assemble virus-like particles to achieve immune competence. Hence, it also serves as a candidate antigen for designing new-generation vaccines.[18] In the present study, analysis of the VP2-deduced amino acid sequence revealed two distinct mutations, S179T and I401V, exclusively identified in isolates from this particular study. These mutations likely signified a new pattern of genetic evolution within FPV strains observed in Aizawl, Mizoram. However, the possible functional impact of these mutations remains unclear and warrants further investigation.

There have been very limited studies conducted on the occurrence, pathology and molecular characterization of FPV in India. The present study is the first comprehensive report on detection and characterization of FPV infection in domestic cat population from North East Region, India. This study identified the FPV as one of the major causes of mortality in domestic cat in North East Region India. The circulating FPV isolates were characterized based on the full VP2 sequences which is crucial for effective disease management and preventive strategies. Identification of two distinct mutations (S179T and I401V) in *VP2* gene that were exclusive to the field isolates from this particular study signified a new pattern of genetic evolution that warrants further investigation to understand the possible functional impact of these mutations.

Acknowledgments

We express our sincere thanks to the Dean, C.V.Sc & A.H., CAU, Selesih, Aizawl, India, for providing the necessary facilities to conduct the research work.

Conflict of interest

The authors declare no competing interests.

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