



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

VET 2024; 9(4): 291-296

© 2024 VET

www.veterinarypaper.com

Received: 08-05-2024

Accepted: 19-06-2024

RL Senthilkumar

Ph.D., Scholar, Department of
Animal Biotechnology, Madras
Veterinary College, Tamil Nadu
Veterinary and Animal Sciences
University, Chennai, Tamil
Nadu, India

TV Meenambigai

Professor and Head, Vaccine
Research Centre-Viral Vaccines,
Centre for Animal Health
studies, Tamil Nadu Veterinary
and Animal Sciences University,
Chennai, Tamil Nadu, India

TMA Senthilkumar

Professor and Head, Zoonoses
Research Laboratory, Centre for
Animal Health Studies, Tamil
Nadu Veterinary and Animal
Sciences University, Chennai,
Tamil Nadu, India

M Madhanmohan

Assistant Professor, Vaccine
Research Centre-Viral Vaccines,
Centre for Animal Health
studies, Tamil Nadu Veterinary
and Animal Sciences University,
Chennai, Tamil Nadu, India

P Azhahianambi

Assistant Professor,
Translational Research Platform
for Veterinary Biologicals,
Centre for Animal Health
Studies, Tamil Nadu Veterinary
and Animal Sciences University,
Chennai, Tamil Nadu, India

Corresponding Author:

RL Senthilkumar

Ph.D., Scholar, Department of
Animal Biotechnology, Madras
Veterinary College, Tamil Nadu
Veterinary and Animal Sciences
University, Chennai, Tamil
Nadu, India

Phylogenetic analysis of *Morbillivirus caprinae* in Tamil Nadu: Lineage IV predominance and variability in gene sequences

RL Senthilkumar, TV Meenambigai, TMA Senthilkumar, M
Madhanmohan and P Azhahianambi

DOI: <https://doi.org/10.22271/veterinary.2024.v9.i4e.1560>

Abstract

Peste des petits ruminants (PPR), the highly contagious, transboundary disease of small ruminants is caused by PPR virus (PPRV) of the *Morbillivirus caprinae* species. As a World Organisation for Animal Health (WOAH) listed disease, cases and outbreaks of PPR are to be notified according to the Terrestrial Animal Health Code. Integrated surveillance and monitoring programmes are crucial to evaluation of disease control strategies as India, along with the world, is marching towards a PPRV free status. We have investigated the presence of PPRV in naive, symptomatic ruminants across different agroclimatic zones of Tamil Nadu. Total 88 suspected samples were screened, detection of the partial Nucleocapsid (N) gene by PCR was confirmatory to the presence of virus, nucleotide sequencing of the locus facilitated lineage deduction and phylogenetic analysis. All the isolates from this study belonged to Lineage IV reiterating the predominance of this lineage in India. Phylogenetic analysis revealed the isolates to be distinct from the outbreak strains of 2004 and 2011 in Tamil Nadu but similar to those from recent incidents in the country. Samples from different agroclimatic zones clustered within a single subclade indicative of an overall closeness, however they were segregated on account of relatedness to isolates from other regions. Virus from Timiri and Tirunelveli, the Northeastern (NE) and Southern zones respectively, were similar to Karnataka, 2023 and Pondicherry, 2022 outbreak strains. Chengalpattu sample, from the NE zone, grouped with isolates from Northern parts of India, whereas Cauvery delta zone PPRV was similar to a Northeastern isolate.

Keywords: nucleocapsid gene, phylogenetic analysis

1. Introduction

Among the major diseases affecting small ruminants, Peste des petits ruminants (PPR) poses a global threat to subsistence farming with an annual economic loss due to mortality. The national control program for PPR, 2011 (PPR-CP) is therefore a forerunner to the global PPR-Global Eradication Program (PPR-GEP) founded by the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (WOAH, formerly OIE) in 2017, but work in consensus towards a singular objective of disease eradication by 2030. Nucleotide sequencing of the causative agent, PPRV, has been an effective tool in disease surveillance, one of the core components of PPR-CP. By providing insights into the evolutionary history of the virus, disease emergence, spread and spillover, it has been critical in decisive strategies towards containment.

Comparative analysis of partial gene sequences encoding the viral Fusion (F) protein laid the foundation for differentiating PPRV into four lineages^[1]. Lineages I & II are African isolates, lineage III is prevalent in Eastern Africa and parts of Arabian Peninsula, whereas lineage IV is dominant across Asia and the Caucasus region^[2, 3]. The pattern of lineage clustering with defined geographic distribution is consistent with Nucleocapsid (N), Matrix (M), Haemagglutinin (H) as well as the large (L) Polymerase^[4] genes of the virus. However, the highly divergent N gene is considered more reliable in the phylogenetic resolution of closely

related strains [5-7].

India reported its first case of PPR in 1987 from the Villupuram district of Tamil Nadu. Though initially restricted to the southern state, the epidemic of 1994 was followed by periodic outbreaks across the country establishing an endemic status [8]. With the exception of a lineage III virus reported in 1992, all the isolates from India belong to lineage IV. The outbreaks have specific spatiotemporal characteristics reflective of the major routes of disease expansion, animal movement on account of grazing, migration or trade and transhumance. Agro-climatic factors and variation in animal husbandry practices too have an impact on prevalence and transmission. According to a recent analysis of transmission dynamics, western and northern India have a larger risk of outbreaks and cases whereas risk of epidemic mortality is high in southern states [9]. Though focal and nationwide mass vaccination campaigns could significantly curtail disease occurrence, lacunae in effective surveillance contribute to unprecedented episodes across the country [10]. India is in the 'control-stage' (Stage 2) of the PPR-GCES milestones, a concerted engagement of all stakeholders in the small ruminant value chain is imperative to achieving a PPR free status [11].

We have investigated the presence of PPRV is non-vaccinated, symptomatic ruminants from different Agroclimatic zones of Tamil Nadu, the North Eastern, North

Western, western, Cauvery delta, southern, high rainfall, and high-altitude zones. The distinction is made on the basis of ecological, geological as well as social features of the area. Nasal swabs from animals exhibiting classical symptoms of PPR infection were analysed for the presence of live virus as well as its genetic material by PCR using N gene primers. Further, genetic characterization of the samples by sequence comparison with isolates from earlier outbreaks across India, as well as other Asian countries available in GenBank database is described. Apart from being a recent survey, a similar investigation of isolates with an expansive geographic distribution within the state of Tamil Nadu has never been undertaken.

2. Materials and Methods

2.1 Sample collection

Nasal samples were collected from eighty eight PPR disease suspected goats and sheep (Figure 1) from different agroclimatic regions of Tamil Nadu state, sampling details are presented in Table 1. None of the animals had a history of vaccination, and were immunologically naïve to the virus. Swabs were transported in 250 µL virus transport medium (VTM, Diagnostic Care Pvt. Ltd.) maintaining proper cold chain to the laboratory and stored in deep freezer (-80°C) until analysis.

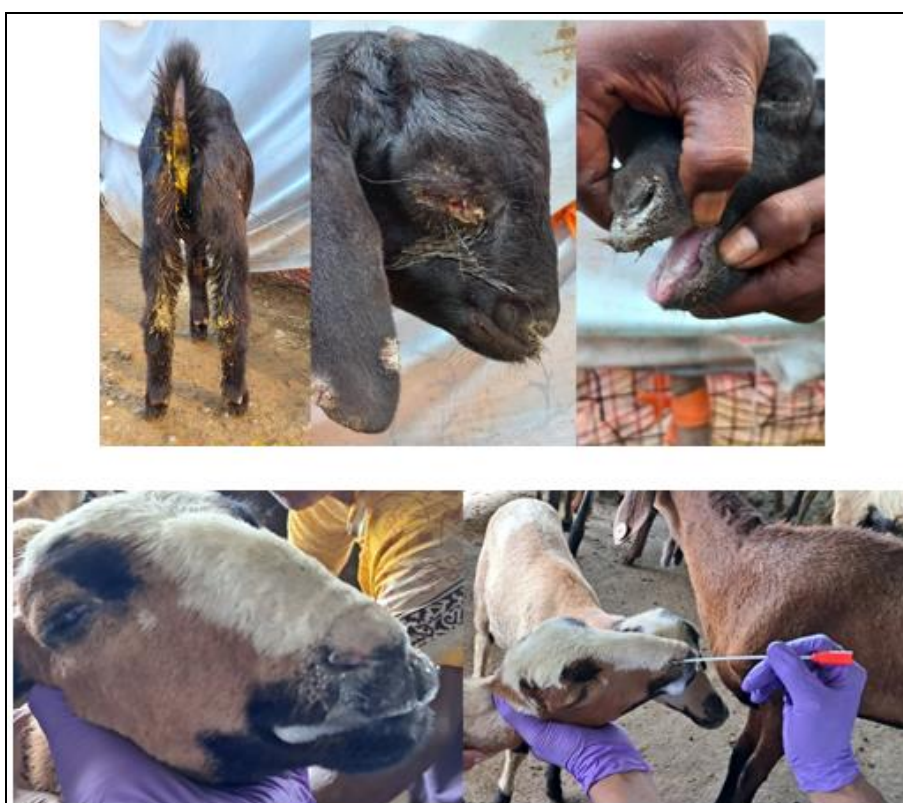


Fig 1: PPR suspected goat & sheep and sample collection

Table 1: Number of samples collected from different agro-climatic zones of Tamil Nadu

S. No.	Zones	No. of samples collected	PPR positive
1.	North-Eastern zone	19	14
2.	North-Western zone	4	1
3.	Western Zone	9	0
4.	Cauvery Delta zone	27	6
5.	Southern zone	22	9
6.	High rainfall zone	7	0
Total samples		88	30

2.2 RNA isolation & cDNA synthesis

The nasal swabs were processed for extraction of viral nucleic acid by TRI reagent (RNAiso Plus, TaKaRa). RNA concentration and purity were determined using a Nanodrop by calculating the ratio of optical density at 260/280 nm and 260/230 nm. cDNA was synthesised using iScript cDNA synthesis kit (Bio-Rad) using 1 µg of RNA as per the manufacturer's protocol. cDNA was stored at -20 °C until further use.

2.3 Polymerase chain reaction & gel extraction

Confirmation of PPR disease was made by PCR assay of the partial N gene of the virus using forward primer 5'-GTCTCGGAAATCGCCTCACAGACT-3' and reverse primer 5'-CCTCCTCCTGGTCCTCCAGAATCT-3' as originally described by Couacy-Haymann *et al*, 2002. The 20 µL reaction contained, 10 µL Taq 2x Master mix RED (Ampliqon, Denmark), 1.5 mM MgCl₂, 1 µL (10mmol/µL) each of forward & reverse primers and 0.6-2 µg of cDNA. The thermal cycling condition was as follows: initial denaturation at 94 °C for 5 min, 40 cycles of denaturation, annealing and extension at 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min, respectively followed by a final extension at 72 °C for 5 min. The PCR products were analysed on a 1% agarose gel with non-carcinogenic nucleic acid stain (MIDORI Green Advance, NIPPON Genetics). The specific amplicon of 352 bp (Figure 2) was excised from agarose gel and extraction was performed using a commercial kit (Qiagen). Extracted PCR products were sequenced by the Sanger's dideoxy chain termination technique (Eurofins Genomics India Pvt. Ltd.).

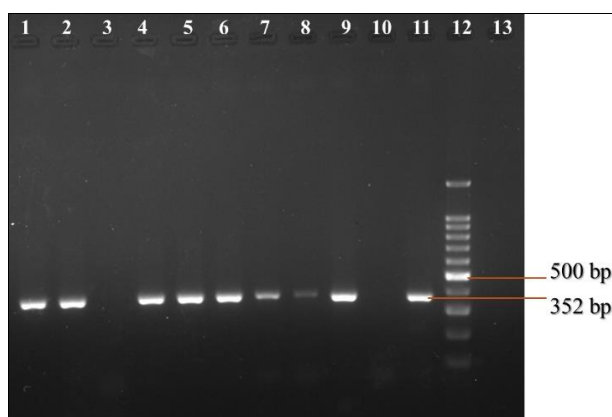


Fig 2: Lane 1-10 field samples, 11- Positive control, 13- Negative control, 12- 100bp DNA ladder

2.4 Nucleotide sequencing and phylogenetic analysis

The N gene nucleotide sequence from samples collected in this study were submitted in GenBank, they were aligned with gene sequences of PPRV from across India, and other geographic regions maintained in the database for phylogenetic characterisation using the MEGA 11 software. Nucleotides 1253 to 1470 of the N gene was used for analysis and Tree construction based on the Neighbour Joining method and Kimura-2 parameter nucleotide substitution model. Bootstrap confidence values were calculated on 1000 replicates.

3. Result and Discussion

3.1 Detection of PPRV in suspected samples by N gene

PCR: All the suspected animals were febrile, presented with oculo-nasal discharges and mouth lesions consistent with the classical symptoms of PPR infection, however out of the 88 samples tested by N gene PCR, only 30 were detected

positive for the presence of virus genetic material. All the samples from the Western and High rain fall zone turned out negative for PPR virus, refer Table 1. The importance of nucleic acid amplification tests in confirming clinically suspicious specimens has been highlighted by several researchers when probable mixed infections confound diagnosis^[12, 13]. PPR is grouped under the *Morbillivirus* genus of the *Paramyxoviridae* family along with genetically similar measles virus (MV), rinderpest virus (RPV), canine distemper virus (CDV), and other phocine, cetacean and feline viruses. The clinical symptoms and progression of PPR, associated with immunosuppression and opportunistic bacterial and parasitic infections, closely resemble and is often confused with those of Rinderpest (RP), or the cattle plague^[14]. The absence of differential diagnosis between RP and PPR has therefore been the major cause for delay in identifying PPRV, and the disease itself as a distinct entity. While the first recorded description of the virus is from Ivory Coast in 1942, the time to most recent common ancestor (TMRCA) of PPRV is estimated as the early 20th century^[15]. The eradication of RP and subsequent divergence and spread of PPR called for major strides in analytical techniques for disease diagnosis that detect specific viral antigens, nucleic acid or antibodies, the latter either induced through natural infection or vaccination^[16].

3.2 Phylogenetic characterisation of isolates

The nucleocapsid region of the PPR genome, as originally described by Couacy-Hymann *et al*, 2002^[17] was also used for lineage classification of the outbreak. Aside of surveillance of the source and route of virus circulation, lineage identification is critical for preparation of homologous vaccine^[18]. The phylogenetic tree constructed using partial N gene sequences distinguished PPRV into 4 lineages. Corroborating with earlier reports, isolates from this study also confirmed the predominance of Lineage IV virus in India, similar to other Asian countries. With the exception of a lineage III virus reported in 1992, all the isolates from India belong to lineage IV. Transhumance and animal trade being attributed to this lineage coexistence in a region, it is also known that lineage IV is expanding geographically and outcompeting pre-existing lineages in different endemic zones of the world^[19, 4, 6]. Interestingly, ancestral PPRV to lineage IV was probably located in India^[15]. As there has been no evidence for virulence associated positive selection in protein coding regions, unchecked movement of infected animals is speculated to be the reason lineage IV expansion^[7].

The dendrogram with Indian isolates alone, (Figure 3) segregated into two major clades, CI and CII, each having 2 subclades, SC I, SCII and SC III, SC IV, respectively. Virus from central, southern, northern, eastern and western parts of India constituted SCI, while SCII comprised of 2 isolates from MP, collected during the year 2004-2005. Tamil Nadu isolates from 2004 and 2011 outbreaks grouped together to form SCIII, whereas those from Tripura, Gujarat, Jhansi and Karnataka formed SCIV. Importantly, the Sungri vaccine strain of North Indian origin was clustered within SCIV. As reported by other researchers, both CI and CII had South Indian and North Indian isolates indicative of disease prevalence sans geographic restriction^[6]. Unlike the study by the Kumar group (2014), we have utilized a greater number of isolates from South India where a region wise clustering of isolates could be observed among some samples. However, few isolates from north and south India grouped together indicating close relatedness.

Clustering of isolates can change based on the target gene as well as geographic distribution of the samples being investigated. We have tried to decipher the phylogenetic

relation of the 2023 Tamil Nadu Isolates, from our study, on a global tree by including N gene-Lineage IV GenBank entries from other Asian countries (Figure 4). African isolates were not included as it is well documented by several research groups [6]. The phylogenetic tree, additionally including all the Asian isolates also segregated into 4 lineages. 3 major clades were formed, namely CI, CII and CIII. Virus from Bangladesh and Tibet along with those from across India formed SC I of CI, while SCII comprising the Sungri vaccine strain among others was similar to SCIV of CII from the previous dendrogram, Fig.1. Virus from Iran and Turkey formed CII, while Tamil Nadu outbreak strains from 2004 and 2011 grouped with a Pakistan isolate to constitute CIII.

Isolates from our study were part of SC I of CI in both the phylogenetic trees. They appear to be significantly different from the strain that was responsible for the 2004 and later 2011 outbreaks in Tamil Nadu (DQ176748 to 51 and KM105872). That the 2023 isolates of the present study grouped with those from more recent outbreaks in

neighbouring states is testimony to the transboundary nature of the disease. Timiri and Tirunelveli (Nort Eastern, NE and Southern zone, respectively) isolates were clustered together and showed more closeness to isolates from Karnataka and Pondicherry, 2019 to 2023. The Orathanadu virus, from the Cauvery delta zone, grouped with the isolate from Mizoram, 2023. Similarly, the Chengalpattu samples (NE zone), from sheep and goat, appeared to be more similar to those from Northern parts of India. Though N sequences from Caprine samples predominate our analysis, similar to our observation researchers have not observed any species-specific lineages or evolutionary groups amongst Indian isolates of PPRV [6]. Despite a wider geographic presence, the genetic diversity of Lineage IV PPRV is on a gradual decline following effective mass vaccination programmes and other control strategies. Presence of multiple clusters of N gene haplotypes within the country is reflective of the transboundary nature of PPR and also of re-emergence of virus from multiple bottlenecks [7].

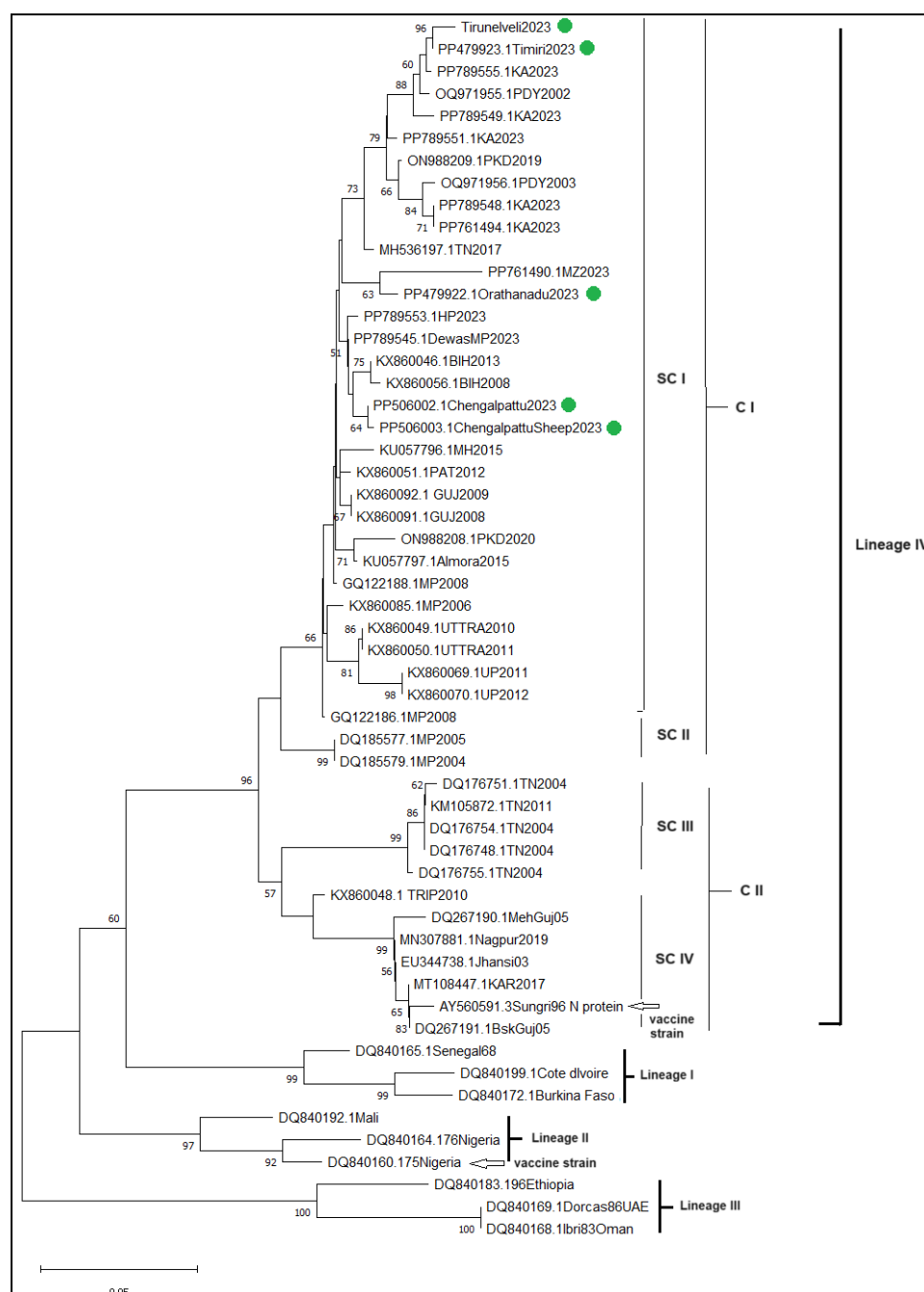


Fig 3: Midpoint-rooted Neighbour-Joining tree based on partial N gene sequences representing the phylogenetic relationship between Tamil-Nadu 2023 isolates (From the present study) with other Indian lineage IV isolates

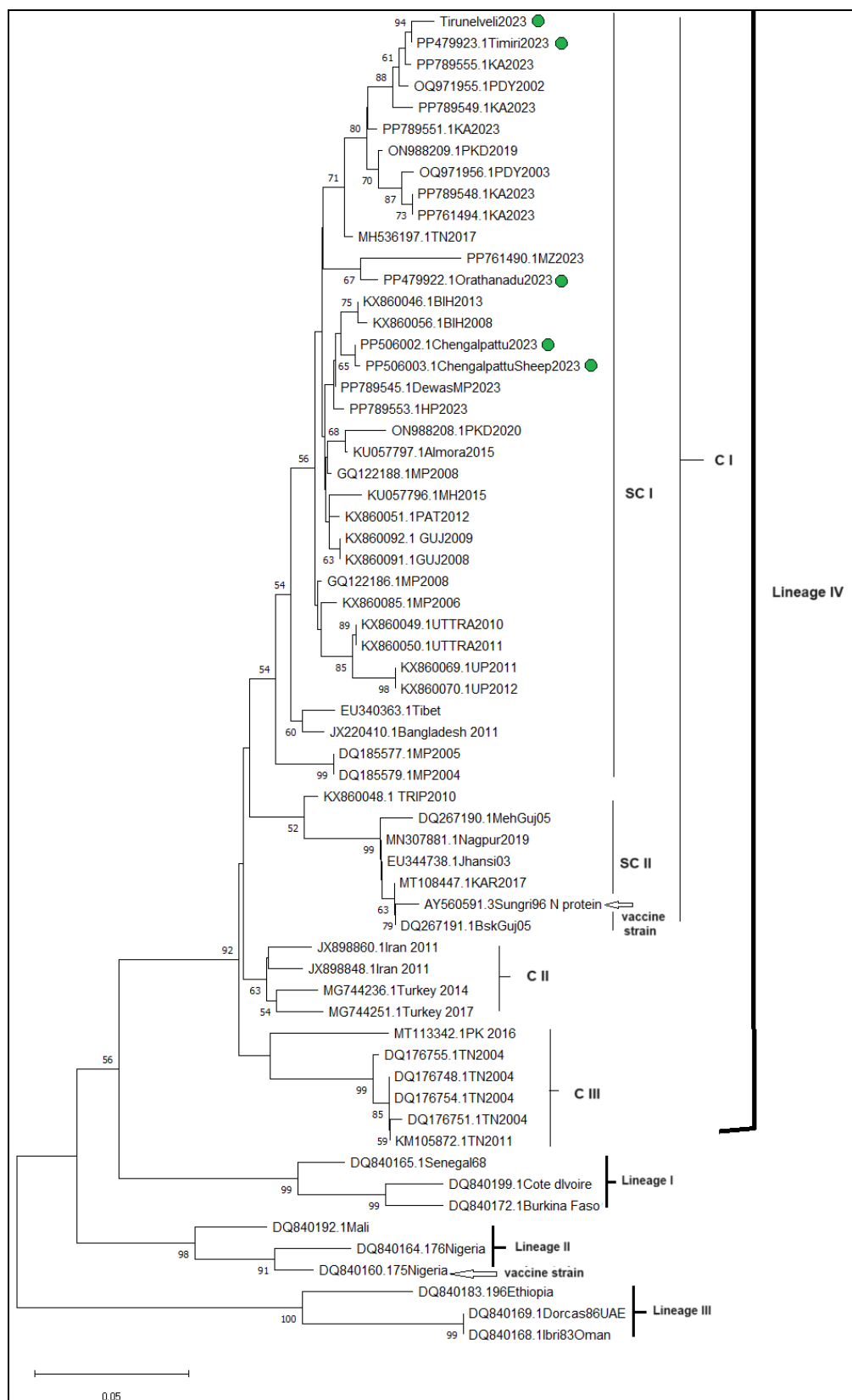


Fig 4: Midpoint-rooted Neighbour-Joining tree based on partial N gene sequences representing the phylogenetic relationship between Tamil-Nadu 2023 isolates (From the present study) with Indian and other Asian lineage IV isolates

4. Conclusion

Given the transboundary nature of PPR, the present isolates reported across Tamil Nadu are probably introduced from recent outbreak episodes in neighbouring states. Distinct from the 2004 and 2011 outbreak strains which were similar to each other, origin of this probably new circulating haplotype cannot be traced without a detailed study. Also, it is to be seen whether the N gene clustering pattern is preserved with F and

H gene loci. Though variability in gene sequences of PPRV lineages is not associated with virulence, nucleotide sequencing is important to understand the geographic trajectory of virus evolution and epidemiological dynamics. Further, stringent biosecurity measures in animal trade and movement can be implemented to check disease spread.

5. Acknowledgement

The authors thank the Tamil Nadu Veterinary and Animal Sciences University for funding and providing all the facilities to carry out this research work, and also thank the ICAR-NCVTCNP for funding.

6. Conflict of Interest: Not available

7. Financial Support: Not available

8. References

1. Shaila MS, Shamaki D, Forsyth MA, Diallo A, Goatley L, Kitching RP, *et al.* Geographic distribution and epidemiology of peste des petits ruminants virus. *Virus Res.* 1996;43(2):149-53.
2. Mahapatra M, Pachauri R, Subramaniam S, Banyard AC, ChandraSekar S, Ramakrishnan MA, *et al.* Ongoing Assessment of the Molecular Evolution of Peste Des Petits Ruminants Virus Continues to Question Viral Origins. *Viruses.* 2021;13(11):2144.
3. Courcelle M, Salami H, Tounkara K, Lo MM, Ba A, Diop M, *et al.* Comparative evolutionary analyses of peste des petits ruminant's virus genetic lineages. *Virus Evol.* 2024;10(1):veae012.
4. Libeau G, Diallo A, Parida S, Evolutionary genetics underlying the spread of peste des petits ruminants virus, *Animal Frontiers.* 2014;4(1):14-20.
5. Kwiatak O, Minet C, Grillet C, Hurard C, Carlsson E, Karimov B, *et al.* Peste des petits ruminants (PPR) outbreak in Tajikistan. *J Comp Pathol.* 2007;136(2-3):111-9.
6. Kumar KS, Babu A, Sundarapandian G, Roy P, Thangavelu A, Kumar KS, *et al.* Molecular characterisation of lineage IV peste des petits ruminants virus using multi gene sequence data. *Vet Microbiol.* 2014;174(1-2):39-49.
7. Padhi A, Ma L. Genetic and epidemiological insights into the emergence of peste des petits ruminants virus (PPRV) across Asia and Africa. *Sci Rep.* 2014;13(4):7040.
8. Balamurugan V, Varghese B, Muthuchelvan D, SowjanyaKumari S, Kumar KV, Dheeraj R, *et al.* Towards eradication of peste des petits ruminants: Post-vaccination evaluation in sheep and goats in Southern Peninsular India. *Virusdisease.* 2020;31(4):539-548.
9. Zhang S, Liang R, Yang Q, Yang Y, Qiu S, Zhang H, *et al.* Epidemiologic and import risk analysis of Peste des petits ruminants between 2010 and 2018 in India. *BMC Vet Res.* 2022;18(1):419.
10. Balamurugan V, Ojha R, Kumar KV, Asha A, Ashraf S, Dsouza AH, *et al.* Post-Vaccination Sero-Monitoring of Peste des Petits Ruminants in Sheep and Goats in Karnataka: Progress towards PPR Eradication in India. *Viruses.* 2024 Feb 22;16(3):333.
11. Legnardi M, Raizman E, Beltran-Alcrudo D, Cinardi G, Robinson T, Falzon LC, *et al.* Peste des Petits Ruminants in Central and Eastern Asia/West Eurasia: Epidemiological Situation and Status of Control and Eradication Activities after the First Phase of the PPR Global Eradication Programme (2017-2021). *Animals* 2022;12(16):2030.
12. Mondal B, Sen A, Chand K, Biswas SK, De A, Rajak KK, Chakravarti S. Evidence of mixed infection of peste des petits ruminants virus and bluetongue virus in a flock of goats as confirmed by detection of antigen, antibody and nucleic acid of both the viruses. *Trop Anim Health Prod.* 2009;41(8):1661-1667.
13. Malik YS, Singh D, Chandrashekar KM, Shukla S, Sharma K, Vaid N, *et al.* Occurrence of dual infection of peste-des-petits-ruminants and goatpox in indigenous goats of central India. *Transbound Emerg Dis.* 2011;58(3):268-273.
14. Roeder, P.L. and Obi, T.U. (1999) Recognizing Peste des Petits Ruminants: A Field Manual. In: Food and Agriculture Organization of the United Nations. Rome. <ftp://ftp.fao.org/docrep/FAO/003/X1703E/X1703E00>.
15. Muniraju M, Munir M, Parthiban AR, Banyard AC, Bao J, Wang Z, *et al.* Molecular evolution of peste des petits ruminants virus. *Emerg Infect Dis.* 2014;20(12):2023-33.
16. Kinimi E, Odongo S, Muyldermans S, Kock R, Misinzo G. Paradigm shift in the diagnosis of peste des petits ruminants: Scoping review. *Acta Vet Scand.* 2020;62(1):7.
17. Couacy-Hymann E, Roger F, Hurard C, Guillou JP, Libeau G, Diallo A. Rapid and sensitive detection of Peste des petits ruminants virus by a polymerase chain reaction assay. *J. Virol. Methods.* 2002;100:17-25.
18. Kumar N, Barua S, Riyesh T, Tripathi BN. Advances in peste des petits ruminants vaccines. *Vet Microbiol.* 2017;206:91-101.
19. Banyard AC, Parida S, Batten C, Oura C, Kwiatak O, Libeau G. Global distribution of peste des petits ruminants virus and prospects for improved diagnosis and control. *J Gen Virol.* 2010;91:2885-2897.

How to Cite This Article

RL Senthilkumar, TV Meenambigai, TMA Senthilkumar, M Madhanmohan, P Azhahianambi. Phylogenetic analysis of small ruminant morbilli virus in Tamil Nadu: Lineage IV predominance and variability in gene sequences. *International Journal of Veterinary Sciences and Animal Husbandry* 2024; 9(4): 291-296.

Creative Commons (CC) License

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.