

REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. Name and designation of ICMR-IF : Dr. Pazhanivel Natesan
Professor
2. Address : Department of Veterinary Pathology
Madras Veterinary College
Chennai-600 007
3. Frontline area of research in which training/research was carried out : Molecular epidemiology and evolution of Rabies virus India; Comparative approaches for molecular, genetic and functional characterization of viruses; Quantitative PCR and Next Generation Sequencing Data Analysis
4. Name & address of Professor and host institute : Dr. Suresh Varma Kuchipudi
Clinical Professor and Head of Microbiology, Associate Director Animal Diagnostic Lab (PADLS- PSU), Department of Veterinary and Biomedical Sciences, Penn State University, Wiley Lane, University Park, PA 16802-1110, Phone: 814-863-5737
Fax: 814-865-3907 Email: skuchipudi@psu.edu
5. Duration of fellowship with exact date : 5½ months 1st Jan to 15th June 2020
6. Highlights of work conducted :

i) Technique/expertise acquired

I learnt several techniques relating to the molecular analyses of rabies viral genomes using MEGA 10 software, BLAST analysis, complete genome sequence analysis, phylogenetic analysis. In addition, I learned molecular techniques including real time PCR, Reverse Transcriptase PCR, cDNA synthesis, nucleic acid sequencing using Illumina and MinION, and standard virological assays including Plaque assay and virus infection studies using a range of cell culture models such as HRT 18G and CHSE. In addition, I completed training in laboratory safety and working with blood borne pathogens.

ii) Research results, including any papers, prepared/submitted for publication

(a) RESEARCH RESULTS MATERIALS AND METHODS

A total of 183 rabies viral sequences retrieved from GenBank were used in this study. The viral sequences originated from a range of SAARC countries including India (78), Afghanistan (14), Bangladesh (9), Sri Lanka (55), Nepal (19), Bhutan (1) and Pakistan (2). In addition, European bat lyssa viruses (1&2), Mokola virus (1), and Duvenhage virus (1) have also been included in the analysis. The nucleotide sequences were aligned using the Clustal W 1.8x program (Thompson et al., 1997). The multiple sequence alignment analysis of N protein amino acid sequences of Rabies virus

was carried out using MEGA X software.

Phylogenetic analysis

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree analyzed from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985).

Distance analysis

The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura and Kumar, 2004) and are in the units of the number of base substitutions per site. This analysis involved 183 nucleotide sequences with 1356 positions in the final dataset. Evolutionary analytical study conducted in MEGA X (Kumar et al., 2018).

RESULTS

Multiple sequence alignment analysis of N protein amino acid sequences

N gene amino acid sequences from several rabies virus isolates from India, Afghanistan, Pakistan, Bangladesh, Bhutan had M60L (Leucine instead of Methionine at 60th position), whereas all the rabies virus isolates from Sri Lanka had Methionine at 60th position (Table 1). Similarly, N gene amino acid sequences of all the isolates from India, Afghanistan, Pakistan, Bangladesh, Bhutan which had M60L (Leucine instead of Methionine) also had S84T, S135P, V179I, A332G, A371T, I379V, T407A, and S448N compared to rabies virus isolates from Sri Lanka. All the Sri Lankan rabies virus isolates had Leucine(L) at 80th position similar to Mokola virus, Duvenhage virus and European bat Lyssa virus 1 & 2, whereas all the other rabies virus isolates had Phenylalanine(F) at 80th position.

Table 1. N gene amino acid sequences from rabies virus isolates from India, Afghanistan, Pakistan, Bangladesh, Bhutan and Sri Lanka

Position	Signatures in India, Afghanistan, Pakistan, Bangladesh, Bhutan	Signatures in Sri Lankan isolates
60	Leucine (L)	Methionine (M)
84	Threonine (T)	Serine (S)
135	Proline (P)	Serine (S)
179	Isoleucine (I)	Valine (V)
332	Glycine (G)	Alanine (A)
371	Threonine (T)	Alanine (A)
379	Valine (V)	Isoleucine (I)
407	Alanine (A)	Threonine (T)
448	Serine (S)	Asparagine (N)

N gene amino acid (AA) sequence of rabies virus originated from Buffalo in India (KU88295) showed truncation at 197th position. Similarly, N gene amino acid of rabies virus isolated from Southern India (MH258823, MH258824, MH258825, MH258826, MH258827, MH258828) showed truncation at 30th position.

Several rabies viruses isolated from Southern India (Tamil Nadu) during the year of 2014 (MH258838, MH258839, MH258840, MH258841, KX434506, KX434507, KX434508) showed AA signatures similar to Sri Lankan rabies virus isolates. All the Sri Lankan isolates had Asparagine at 448th position instead of Serine in other isolates. Southern Indian isolates KX434506, KX434507, KX434508 also had the S448N.

Some of the Southern Indian isolates (MH258838, MH258839, MH258840, MH258841) showed Serine at 119th position instead of Asparagine, whereas MH258839, MH258840 also had Arginine at 29th and 33rd position instead of Lysine (K) and Alanine (A), respectively, and Glutamic acid (E) at 38th position instead of Lysine (L).

In addition, several single amino acid polymorphisms were observed in Southern Indian rabies virus isolates. MH258839, MH258840 had K29R, A32R, K38E, N119S, F438S and A439P. MH258839, MH258840, MH258841, MH258842 showed A404P, R418I, S447C and S448N. All the rabies virus isolates from Southern India during 2014, uniquely showed N443S compared to other sequences.

Phylogenetic analysis

Phylogenetic analysis based on amino acid sequences of n protein of rabies virus

Phylogenetic analysis based on N protein AA sequences revealed Rabies viruses from India, Afghanistan, Bangladesh, Sri Lanka, Nepal, Bhutan, and Pakistan formed a single group (single cluster). There was no distinct grouping based on geographical location (Figure-1 Phylogenetic tree).

Phylogenetic analysis based on nucleotide sequences of n gene of rabies virus

Phylogenetic analysis based on nucleotide sequences revealed Rabies viruses from India, Afghanistan, Bangladesh, Sri Lanka, Nepal, Bhutan, and Pakistan formed a single group (single cluster). There was no distinct grouping based on geographical location.

Distance analysis

Pairwise distance analysis showed India, Afghanistan, Bangladesh, Nepal, Bhutan and Pakistan were in same identity at the level of 0.00 to 0.07 (93 to 100%). But Sri Lankan accession numbers revealed distance to other countries at the level of 0.15 (85%). Overall mean pairwise distance was 10 per cent.

References

- Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**:783-791.
- Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* **35**:1547-1549.
- Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**:406-425.
- Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* **101**:11030- 11035.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876–4882.

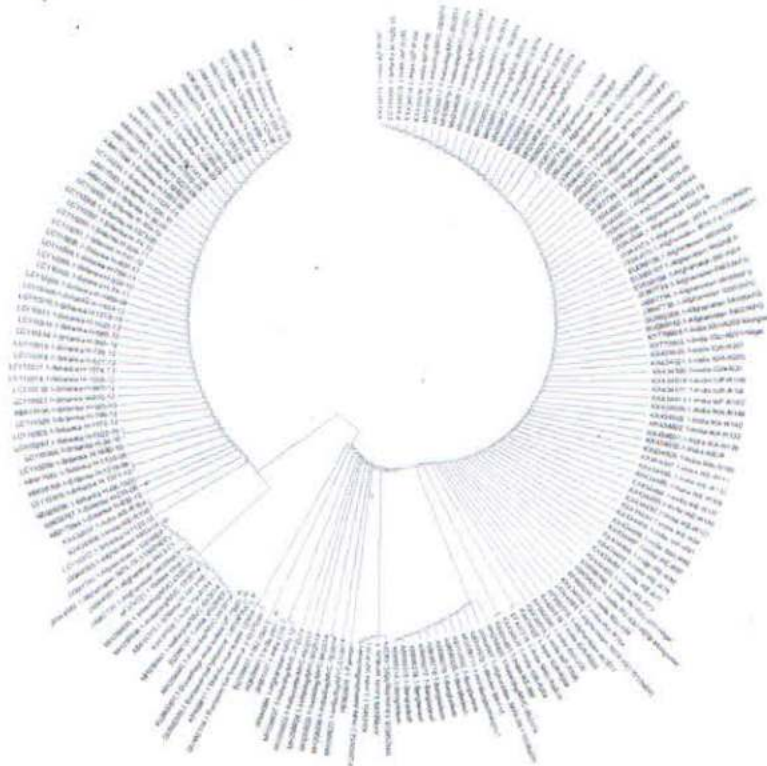


Figure 1. Phylogenetic analysis based on amino acid sequences of n protein of rabies virus

(b) PAPERS PREPARED/SUBMITTED FOR PUBLICATION:

Prepared

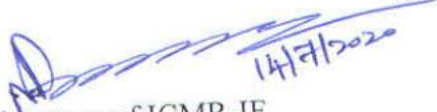
1. Phylogenetic analysis of rabies virus N gene of SAARC countries with India
2. Genomic analysis of Bovine viral Diarrhea virus isolates of United States
3. Nucleic acid sequencing of Bovine corona virus of United States

Submitted

1. Meera Surendran Nair, Lingling Li, Ruth H Nissly, Victoria S Cavener, Michele Yon Rhiannon Barry, Pazhanivel Natesan, Nagaraja Thirumalapura, Deepanker Tewari, Bhushan M Jayarao and Suresh V Kuchipudi (2020). Whole genome sequences of two *Streptococcus equi* subsp. *zooepidemicus* isolates associated with swine mortality in Pennsylvania. Submitted to *Microbiology Resource Announcements*.

iii) Proposed utilization of the experience in India :

Based on the experience gained from the Pennsylvania State University, I will continue to do further research on Rabies in India for the rabies control program. I plan to submit a research proposal relating to molecular epidemiology and evolutionary analyses of rabies viruses in India for funding to Indian Council of Medical Research and/ Department of Science and technology.

 14/7/2020
Signature of ICMR-IF

**Professor,
Dept. of Veterinary Pathology,
Madras Veterinary College,
Chennai-600 007.**

ICMR Sanction No.
No INDO/FRC/452/S-58/201 9-20-IHD Dated: 19th December, 2019 of the Assistant Director
General (A), Dept. of Health Research (Ministry of Health & Family Welfare),
V. Ramalingaswami Bhawan, Ansari Nagar, New Delhi-110 029