LETTER TO THE EDITOR

PHYLOGENETIC CHARACTERIZATION OF RABIES VIRUS ISOLATES FROM CHENNAI, INDIA

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Rabies is an important zoonotic disease that still represents a serious problem in many developing countries. Human mortality from endemic canine rabies was estimated to be 55,000 deaths per year worldwide and 56% of that in Asia. Rabies occurs in all parts of Indian subcontinent except for Andaman, Nicobar and Lakshwadeep group of islands. Dogs are responsible for 96% of human rabies cases. The mortality in livestock and pet animals is far higher than that officially estimated due to lack of exact reports (*1*).

Rabies virus (RV) belongs to the species *Rabies virus,* the genus *Lyssavirus*, the family *Rhabdoviridae*. The nucleoprotein (N) gene of RV is the most popular target for rabies diagnosis by RT-PCR because it is highly conserved in lyssaviruses and can be employed for their genetic analysis (*2–5*). The N gene, although highly conserved, allows viral strains to be accurately differentiated by analyzing genetic differences present within the gene (*6*). Molecular epidemiology of rabies has been performed not only at regional but also at global level (*7–8*). However, there is little information available on the molecular epidemiology of RV in Asia (*9–12*). Regarding Indian RV isolates, we have previously reported a full-length N gene sequence of an isolate (*13*).

In this study, to characterize epidemiology of RV in Chennai, the capital of Tamil Nadu state, we sequenced a portion of the N gene (nt 311–710) of nine RV isolates obtained from 7 dogs, one calf and one goat. Brain specimens originated from necropsy of suspected rabies animals, which was performed at the Madras Veterinary College Hospital, Chennai, in 2003–2005. Rabies diagnosis was done by a direct immunofluorescence (IF) test on impression smears of hippocampus region of the brain (*14*) using a conjugate of polyclonal antibody to RV N protein (Bio-Rad, Australia) The IF test-positive samples were subjected to RT-PCR. Total RNA was extracted using TRIZOL® reagent (Invitrogen) following the manufacturer's instructions. cDNA was prepared from 1 µg of total RNA using the THERMOSCRIPT ™ RNase H-reverse Transcriptase Kit (Gibco-BRL, USA) and random hexamer primers. In a nested PCR, two different primer pairs were used: NF28 (5'-GCGGATCCCACCTCTACAATGG ATGCCG-3', nt 61–80) and NF32 (5'-TTATGAGTCA CTCGAATATGTCT-3', nt 1423-1401), and NF1 (5'-GACATGTCCGGAAGACTGG-3', nt 319–337) and NR1 (5'-GTATTGCCTCTCTAGCGGTG-3', nt 823–842), internal to the first primer pair The reaction was performed in MJ Research Thermal Cycler (PTC-200). It consisted of 5 cycles of 94°C/2 mins (denaturation), 45°C/1 min (annealing), and 72°C/2 mins (elongation). The annealing temperature was increased to 50°C in further 5 cycles and to 55°C in further 20 cycles. Final elongation consisted of 72°C/10mins.

The PCR products were purified using a gel extraction kit (AuPreP, USA) and sequenced using the primer NR1 in an ABI Prism 3700 DNA Sequencer (Applied Biosystems, USA). The obtained nucleotide sequences were deposited in GenBank. To compare the sequenced Indian RV isolates with

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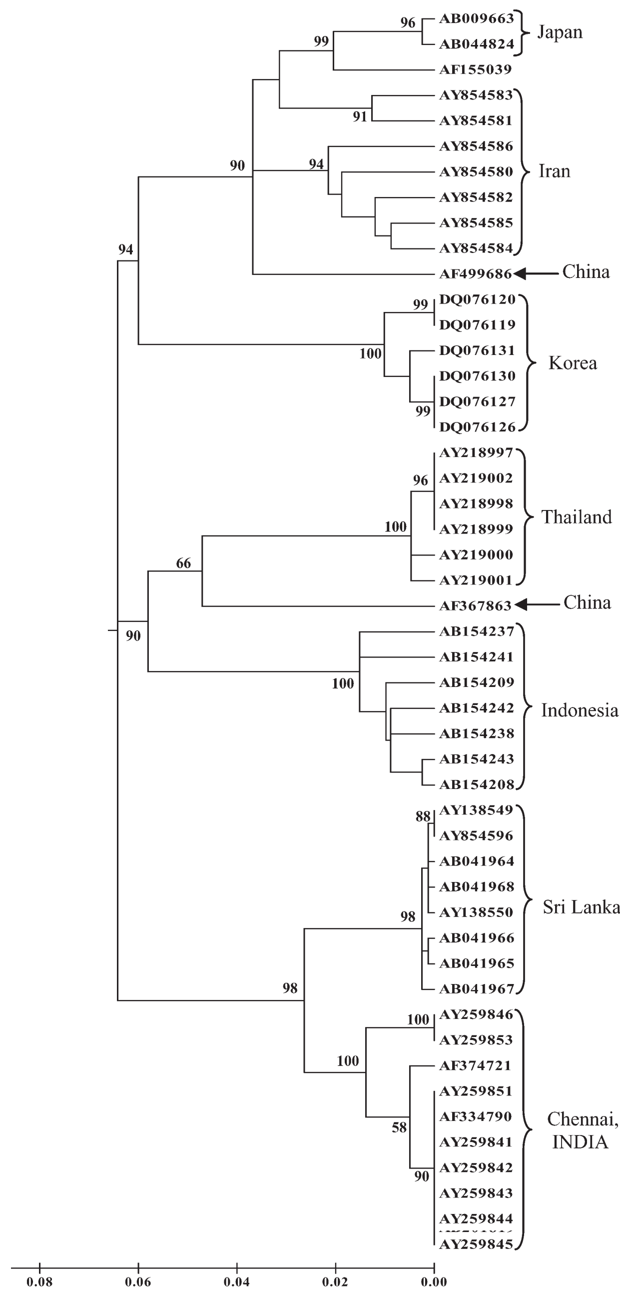
Abbreviations: IF = immunofluorescence; N = nucleoprotein; RV = Rabies virus



RV isolates from other countries the respective sequences deposited in GenBank were employed. Multiple alignment was done using MEGA 3.1 (*15*) and Clustal W programs. Phylogenetic tree was constructed by the neighbor-joining method and 1000 replications were done for bootstrap analysis.

In phylogenetic tree (the figure), all the Chennai RV isolates clustered together with those from Sri Lanka, confirming their genetic relatedness and suggesting a possibility of their common ancestor. This kind of conclusion has been drawn already earlier for one Chennai RV isolate (*13*). In general, RV isolates from India and Sri Lanka appear to be closely related (*16–17*).

It was also interesting to find out that, in the tree, the isolates from Iran, a Middle East country, clustered together with those from Japan and Korea, Far East and South East countries. However, other South East countries like Thailand and Indonesia also clustered together.

Further studies on greater number of isolates from various parts of India will be required for gaining a more precise knowledge of molecular and epidemiological characteristics of RV strains circulating in India. Such knowledge will be useful for tracing the routes of rabies infection and for establishing measures of eradication of rabies.

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