

Molecular characterization of rabies virus of Nepal

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Abstract

A study was performed to identify the Rabies Virus genomes circulating in Nepal in 2009. Three brain samples, collected from carcass of buffalo, human and dog infected with Rabies were sent to Pasteur Institute, in France for molecular characterization and identification. The entire nucleoprotein genes (NG) of these samples were sequenced and analyzed phylogenically. Sample from buffalo was identified as Indian subcontinent lineage where as samples from human and dog were identified as Arctic lineage of Rabies virus.

Introduction

Rabies is a fatal viral zoonotic disease which affects all warm blooded living beings. It is recorded as an endemic disease in Nepal. It is spread all over country as well as occurs through out the year. The incidence of Rabies has been reported in bovine, caprine, ovine, canine and equine species of animals. Over the past five years (January 2005 to December 2009) 411 outbreaks and 700 death of animal has been recorded in 45 districts of Nepal (Veterinary Epidemiology Centre, 2010). Laboratory diagnosis of Rabies is only limited to Central Veterinary Laboratory (CVL), Kathmandu and disease been confirmed in human, dog, buffalo, cattle, sheep, goat and mice. A total number of 149 samples were submitted to CVL for laboratory confirmation from July 16, 2004 to July 15, 2009 and 66% were positive (Annual Technical Report 061-066). Both Sylvatic and urban epidemiological cycles of Rabies virus exist in this country giving potential risk from jackal, fox and dog respectively. Dog is considered as a principal reservoir or vector and also 99% responsible to transfer disease in human. Rabies is the cause of death for 100 people and 200 animals every year in this country. Therefore 35,000 people get post exposure treatment against rabies. Rabies Vaccine production Laboratory, Kathmandu has been producing Tissue Culture Anti Rabies Vaccine (TCARV) for animal use in the country since 2006 however it produces only 20,000 doses of vaccine which is not enough to meet the national need. This disease is panic and spread all over the country therefore molecular epidemiological study is essential to identify the genome of Rabies virus existing in the country. This article is preliminary study of molecular characterization of rabies virus in Nepal.

Material and Methods

Sample collection and dispatch

Three brain samples infected with rabies virus were submitted at Central Veterinary Laboratory for confirmation from different institutions in different time. Human brain was collected from Lalitpur by Patan Hospital on 30/07/2003 from 12 years school girl after death. Buffalo brain sample was collected from Kaski by Regional Veterinary Laboratory, Pokhara on 11/07/2003. Dog brain sample was collected from Kathmandu by CVL on 08/09/2008. All of those samples were tested at CVL by performing Negri body test, FAT and, mice inoculation and also Rapid test as soon as possible and found positive for Rabies (Annual Technical Report, 2060/061). The portion of positive samples were provided to Rabies Vaccine Production Laboratory on request and, divided into several containers and stored at -80° C until use. A part of those samples were dispatched to Pasteur Institute, France according IATA instruction 602 on July 21, 2009.

Molecular characterization

Total RNA was extracted from clinical samples using TRIzol or by using commercial RNA extraction kits. Standard reverse transcription polymerase chain reaction (RT-PCR) was used to amplify the complete N gene. PCR product were purified and sequenced with automated sequencing system and finally phylogenetic analysis was performed by following the method described by Bourhy *et al.*, 2008.

Result

All three samples were positive Rabies viral RNA in RT-PC. Dog and human brain sample were detected as Arctic lineages of Rabies where as buffalo brain was identified as Indian subcontinent lineage on phylogenetic analysis. The sequences of RABV of dog, human and buffalo are given below.

Dog_Nepal_Nucleoprotein_Complete

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>ATGGATGCCGACAAGATTGTATTCAAAGTCAATAATCAGGTGGTCTCTTT
GAAGCCTGAGATTATCGTGGATCAATATGAGTACAAGTACCCTGCTATCA
AGGACTTGAAGAAGCCCAGTATCACCCCTAGGGAAAGCCCCCGATTTGAAC
AAGGCATACAAGTCAGTCTTATCAGGTTTGAATGCTGCCAAGCTTGATCC
TGATGATGTATGTTCTACTTGGCAGCTGCGATGCAGTTCTTCGAGGGGA
CATGTCCTGAAGACTGGACCAGCTATGGGATCTTGATTGCACGAAAAGGA
GATAAGATCACCCCGGATTCTCTTGTGGAGATAAAGCGTACTGATGTAGA
AGGGAATTGGGCTTTGACGGGGGGGATGGAAGTACGAGGGACCCCACTG
TTCCTGAGCATGCGTCTTTAGTCGGTCTTCTCTTGAGCCTGTATAGGCTG
AGCAAGATATCTGGGCAAAACACCGGTAACATAAAACAAATATTGCAGA
TAGGATAGAGCAGATTTTCGAGACAGCCCCTTTTATTAATCGTAGAAC
ACCATACTCTAATGACAACTCACAAGATGTGTGCCAATTGGAGTACGATA
CCAACTTCAGATTCTTGGCAGGGACCTACGACATGTTTTCTCTCGGAT
TGAGCACCTGTATTCAGCGATTAGAGTAGGCACAGTAGTCACTGCTTATG
AGGACTGCTCGGGGCTGGTGTCTGTTTACTGGGTTTCATAAAACAGATAAAT
CTCACTGCAAGGGAAGCAATACTGTATTTCTTCCACAAGAACTTCGAGGA
AGAAATAAGAAGAATGTTTGAGCCAGGGCAAGAGACAGCTGTTCTCACT
CCTATTTCACTTCCGTTCACTGGGCTTGAGTGGAAGTCCCCCTTAT
TCATCAAATGCAGTTGGTCATGTGTTCAATCTCATTCACTTTGTTGGATG
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TTATATGGGGCAAGTAAGGTCTCTGAATGCAACGGTCATTGCTGGATGTG
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ATGGAACGTGCAATTCAGATGATGAGGACTACTTCTCCGGTGAAACCAGA
AGCCCCGAAGCTGTTTATGCCCCGAATCATGATGAACGGAGGCCGACTAAA
GAGATCGCACATACGGAGATATGTTTCTGTCAGCTCCAATCACCAGGCTC
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TGA

Human_Nepal_Nucleoprotein_Complete

>ATGTAACACCTCTACAATGGATGCCGACAAGATTGTATTCAAAGTCAATA
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GTAGTCACTGCTTATGAGGACTGCTCGGGGCTGGTGTGCTTTACTGGGTT
CATAAAACAGATAAATCTCACTGCAAGGGAAGCAATACTGTATTTCTTCC
ACAAGAACTTCGAGGAAGAAATAAGAAGAATGTTTGAGCCAGGGCAAGAG
ACAGCTGTTCCCTCACTCCTATTTCACTTCCGTTCACTGGGCTTGAG
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TTCATTTTGTGGATGTTATATGGGGCAAGTAAGGTCTCTGAATGCAACG
GTCATTGCTGGATGTGCTCCTCATGAGATGTCTGTCTTAGGGGGCTATTT
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ACGGAGGCCGACTAAAGAGATCGCACATACGGAGATATGTTTCTGTCAGC
TCCAATCCCCAGGCTCGTCCGAACCTCATTTGCCGAGTTTCTAAACAAAAC
GTATTCCATTGATTAATGAAAGGC

Bovine_Nepal_Nucleoprotein_Complete

>ATGGATGCCGACAAGATTGTATTCAAAGTTAATAATCAGGTGGTCTCCTT
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AAGGCATACAAATCTGTTTTGTCGGGCATGAATGCTGCCAAGCTTGACCC
TGATGATGTGTGCTCCTATTTAGCAGCTGCAATGCAATTTTTTCGAGGGAT
CCTGTCCTGAGGACTGGACTAGCTATGGAATACTGATCGCACGGAAAGGG
GACAAGATCACTCCAGATTCTCTCGTAGATATAAAGCGCACTGATGTAGA
GGGAATTGGGCCCTGACCGGAGGAATGGAATTAACGAGGGACCCCACTG
TTTCTGAGCATGCATCTCTAGTCGGTCTTCTTTTGAGTCTGTATCGGTTG
AGCAAAATATCGGGGCAAAACACCGGCAATTACAAGACAAACATTGCAGA
TAGGATAGAGCAGATTTTTGAGACAGCCCCTTTTGTCAGATTGTGGAAC
ACCATACTTTGATGACGACTCACAAGATGTGTGCTAATTGGAGTACCATA
CCGAACTTCAGATTCTTAGCTGGAACATACGACATGTTTTTCTCCCGGAT
TGAGCATCTGTATTCAGCAATTAGAGTAGGCACAGTTGTTACTGCTTATG
AGGACTGCTCAGGGCTGGTGTCTGTTTACAGGGTTCATAAAGCAAATAAAT
CTTACTGCAAGAGAAGCGATACTGTATTTCTTCCACAAGAACTTTGAGGA
GGAGATAAGGAGAATGTTTGAGCCAGGTCAGGAGACAGCTGTTCTCATT
CCTATTTCAATTCATTTCGTTTCAATGGGCCTAAGCGGTAAAGTCCCCGTAT
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TTATATGGGTCAAATCAGATCTCTTAATGCAACGGTTATTGCTGCATGTG
CCCCTCATGAGATGTCTGTTCTCGGGGGATATCTAGGGGAAGAGTTTTTT
GGAAAGGGGACATTTGAAAGAAGATTCTTCAGAGATGAGAAAGAACTTCA
AGAATACGAGGCGGCTGAACTGACGAAGACTGACATTGCATTGGCTGATG
ACGGAACCGTCAATTCTGATGACGAGGATTATTTCTCAGGTGAAACCAGG
AGTCCTGAAGCTGTTTATACTCGAATCATGATGAACGGAGGTGATTGAA
AAGATCACACATAAGGAGGTATGTTTCAGTCAGTTCCAATCATCAAGCTC
GCCCAAATCATTCGCAGAGTTTCTCAACAAGACGTATTCTAGTGACTCA
TAA

Discussion

Dog associated RABV forms distinct phylogenetic group that has six major clusters identified as the Africa 2, Africa 3, Arctic-related, Asian, Cosmopolitan and Indian subcontinent clades. Among these 6 clades of RABV Arctic and Indian subcontinent lineage were identified in Nepal in this study. Although Arctic clade of RABV was also identified in Nepal in 1998 in 6 brain samples collected from one sheep, four dogs and one mongoose from Kathmandu (Personal Communication with Dr. J.N. Rai, Consultant, Rabies Vaccine Production Laboratory, Tripureshwar, Kathmandu). The Arctic-related clade has also been isolated from dog, raccoon dog, arctic fox, red fox, striped skunk and wolf. It is circulating in Russia, Nepal, North India, Korea, Greenland and North America (Hyun *et al*, 2005). However Indian subcontinent clade was first identified in Nepal in 2009. Therefore this is the first report that provides scientific evidence for the presence of Indian Subcontinent clade circulating in buffaloes at Kaski district of Nepal. Indian Subcontinent clade of RABV was considered to be distributed only within Southern India and Sri Lanka (Nanayakkara *et al*, 2003) to date. This is very important finding because it is notable because it is one of the first to diverge. The status of other lineages of RABV is known as limited number of samples was tested therefore there is enough room to continue this study to identify different lineages of RABV circulating in different parts of country in different species of animal.

Conclusion

Arctic and subcontinent lineages have been found circulating in Nepal to date.

Acknowledgement

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