*Neurology Asia 2009; 14 : 67 – 69*

# Genetic characterization of a Nipah virus isolated from a *Pteropus vampyrus* in Malaysia

[1]SH Sharifah, [2]AR Sohayati, [2]M Maizan, [3]LY Chang, [2]M Sharina, [2]AK Syamsiah, [4]K Latiffah, [4]SS Arshad, [2]CM Zaini, [5]F Humes, [5]P Daszak, [5]J Epstein

*[1]School of Medicine and Health Sciences, Monash University Sunway Campus, Malaysia; [2]Veterinary Research Institute, Department of Veterinary Services, Malaysia; [3]Faculty of Medicine, Universiti Teknologi MARA, Malaysia; [4]Veterinary Faculty, Universiti Putra Malaysia; [5]The Consortium for Conservative Medicine, New York, USA*

### *Abstract*

Sequence and phylogenetic analyses of the N, P, M, F, G and L open-reading frames of a Nipah virus isolated from a *Pteropus vampyrus* illustrated the uniqueness of the genetic signature of this virus compared to all the other Malaysian isolates of Nipah virus from pigs, bat (*Pteropus hypomelanus*) and humans, as well as the Nipah virus isolated from *Pteropus lylei* in Cambodia, and that from human in Bangladesh. The Nipah virus of *P. vampyrus* is more closely related to the Nipah virus isolate from *P. lylei,* Cambodia than to Nipah virus human isolate of Bangladesh.

## INTRODUCTION

Nipah virus (NiV), is a zoonotic virus belonging to the family *Paramyxoviridae* and the genus *Henipavirus* that caused severe respiratory disease in pigs and fatal acute encephalitis in humans.[1] Substantial serological and virological evidences have demonstrated that bats of the *Pteropus* sps. are the natural host and reservoir of this virus. NiVs have been isolated from the urines of *Pteropus hypomelanus* (Island flying foxes) at roost[2] in Malaysia and *Pteropus lylei* at roost in Cambodia.[3] Recently, for the first time, a NiV suggested to be a recrudesced virus, was isolated from the urine of a captive female *Pteropus vampyrus*.[4] The bat was initially seropositive, turned seronegative after 20-26 days, and remained in this state for 11 months before NiV was isolated when the antibody was on the verge of rising, that is at a serum neutralizing test (SNT) titer of 4. The bat seroconverted 2 weeks later. The success of the isolation was based on the hypothesis that naturally infected bats may be persistently infected and may harbor the virus as to such a time that it is conducive for the virus to be reactivated. In this brief communication, genetic characterization and relationship of the newly isolated NiV from *P. vampyrus* with other known available NiVs in GenBank are presented.

## METHODS

Reverse transcriptase-PCR products of the open-reading frames (ORFs) of the N, P, M, F, G and L genes of the fourth passage virus in Vero cell cultures were submitted to Medigene Company, Singapore, for direct forward and reverse sequencing. The nucleotide sequences were edited using the BioEdit sequence alignment editor version (BioEdit, Ibis Biosciences, USA). Further analysis of the nucleotide and amino acid sequences were conducted using the ClustalX multiple alignment program version 1.83.[5] Phylogenetic analyses were generated by neighbor-joining method. The sequences were analysed together with all the other NiV sequences available in GenBank of the following isolates; NiV isolates from pigs in Malaysia (AJ627196: Tambun, AJ564621: Sg. Buloh, AJ564622: Seremban), humans in Malaysia [AJ564623 (UM0128), AF212302(human isolate sequenced by Centre For Disease Control , Atlanta, USA); AY029767 (UMMC1), AY029768 (UMMC2)], *P. hypomelanus* of Tioman, Malaysia (AF376747), *P. lylei* of Cambodia (N gene-AY858110 and G gene-AY858111) and human in Bangladesh (AY988601).

## RESULTS

Alignment of amino acid sequences showed high sequence similarities (99%) between N, F and G proteins of NiV *P. vampyrus* with all the six Malaysian NiVs sequences (NiV-hypomelanus, NiV pig-Tambun, NiV pig-Seremban, NiV pig-Sg. Buloh, NiV humans-UMMC1, UMMC2). However, a more detailed analysis showed that NiV *P. vampyrus* differed from all known Malaysian

NiVs at 50 nucleotide and 26 amino acid positions. Twenty of these amino acid changes occur in the highly phosphorylated deduced P protein where substitutions with proline occur at seven amino acid positions. Five proline substitutions occur in the 321 amino acid carboxyl terminal region (amino acid positions 430, 437, 438, 454 and 467) and the other two (amino acid positions 175 and 206) occur in the 138-389 amino acid region of the P protein. These proline residues might result in significant changes in the structure and functions of the P protein. In the deduced G protein of the NiV *P. vampyrus*, a substitution of N481D resulted in the loss of one N-linked glycosylation site compared to the deduced eight potential N-linked glycosylation sites present in all the other Malaysian NiV isolates. In the G proteins, all the residues especially those of the eight N-linked glycosylation sites, cysteines and proline residues seemed to be well conserved. There is only one amino acid substitution from leucine to glutamate at residue 470. In the deduced N protein, for all the Malaysian NiVs compared, no changes occurred in the 29 amino acids C-terminal region of the 468-496 amino acids of the N protein, which is involved in the binding to the P protein. In the deduced F protein, three amino acid substitutions occurred i.e. C11S, P63A, and I400K. The F protein is predicted to be a Type 1 transmembrane protein, with a membrane spanning domain located at 489-518.[6,7] The Fo, F1, F2, the predicted cleavage site F1 (cleavage at arginine 109), and the predicted six N-glycosylation sites[6,8] are well conserved in all the Malaysian NiVs including the NiV *P. vampyrus*. However, a cysteine-serine change at amino acid position 11 was noted, which may have an effect on the structure of the protein. The M and L proteins are well conserved with only one amino acid change in the M proteinof the NiV *P. vampyrus,* i.e. A354G.

The N amino acid sequence of *P. vampyrus* shares 98% identity with both of NiV *P. lylei*, Cambodia and NiV human-Bangladesh strain. On the other hand, the G amino acid sequence of NiV *P. vampyrus* share 98% identity with NiV *P. lylei*

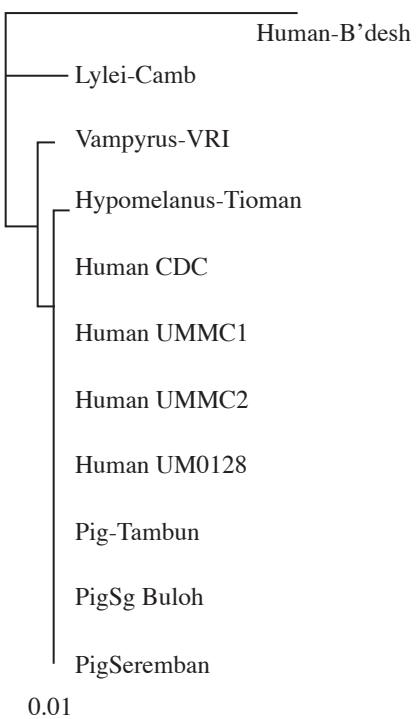


Figure 1. Phylogenetic analysis of the 1,599 nucleotides of the ORF of the N gene illustrating the relationships of the *P. vampyrus* Nipah virus isolate to all other known NiVs. These NiVs are: pigs of Malaysia from Tambun, Sg. Buloh and Seremban; humans of Malaysia-UMMC1, UMMC2 and UM0128; human of CDC, Atlanta, USA (Malaysian Niv isolated during the outbreak of 1998/9); .*P. hypomelanus* of Tioman, Malaysia; *P. lylei* of Cambodia and human of Bangladesh. The scale of 0.01 represents 1% amino acid sequence divergence.

and only 95% identity with NiV from human-Bangladesh strain. The F amino acid sequence of NiV *P. vampyrus* shares 98% identity with that of human-Bangladesh strain. The P amino acid sequence of NiV *P. vampyrus* shares 96% identity with all the six Malaysian NiVs. However the P protein of NiV *P. vampyrus* shares only 90% similarities with NiV human-Bangladesh strain.

## DISCUSSION

Based on the N gene, phylogenetic analysis showed that all the Malaysian isolates of NiVs from pigs and human, Malaysian NiV isolate of human sequenced by CDC, Atlanta, USA, and from *P. hypomelanus* clustered tightly together, as compared to the NiV *P. vampyrus* which diverged from the cluster. The NiV *P. lylei,* Cambodia and NiV human-Bangladesh formed separate branches all together (Figure 1). Phylogenetic analysis conducted with the sequences of other genes and proteins of NiV *P. vampyrus* produced similar results. The findings, therefore, implied that this NiV isolated from *P. vampyrus* is indeed a new strain. Differences in the amino acids of each of the genes may play pivotal roles that might be related to the structure and functions of each of the proteins involved in viral infectivity, viral persistence and recrudescence.

## REFERENCES


1. Chua KB, Bellini WJ, Rota PA, *et al*. Nipah virus: a recently emergent deadly paramyxoviruses. *Science*. 2000; 288:1432-5.
2. Chua KB, Koh CL, Hooi PS, *et al*. Isolation of Nipah virus from Malaysia Island flying foxes. *Microbes Infect*. 2002; 4:145-51.
3. Reynes JM, Counor D, Ong S, *et al*. Nipah virus in Lyle's Flying Foxes, Cambodia. *Emerg Infect Dis* 2005:11; 1042-7.
4. Sohayati, AR. Endemicity of Nipah virus in *Pteropus* sps. in Peninsular Malaysia. PhD. thesis Universiti Putra Malaysia, Serdang, Malaysia, 2008 (Submitted).
5. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins, DG. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; 24: 4876-82.
6. Harcourt BH, Tamin, A, Ksiazek TG, *et al*. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. *Virology* 2000; 271:334-49.
7. Wang LF, Harcourt BH, Yu M, *et al*. Molecular Biology of Hendra and Nipah viruses. *Microbes Infect*. 2001; 3:279-87.
8. Harcourt BH, Lowe L, Tamin A, *et al*. Genetic characterization of Nipah Virus Bangladesh, 2004. *Emerging Infect Dis*. 2005; 11:1594-7.