

Research Article

Selection of a Rabies Virus Strain as a New Candidate for Vaccine Development in Thailand

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Abstract

Rabies remains a severe viral zoonotic disease worldwide is always fatal once clinical symptoms appear in human and domestic animals. Furthermore, the Rabies virus is commonly transmitted to humans by infected domestic animals. There is no cure available and vaccination is the only way to prevent virus infection. The virus actively circulates in Thailand with several fatal cases and outbreaks in animal; mostly dogs have been recorded for the past decade. Additionally, Thailand import 2.5 Million doses of rabies vaccine

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yearly, the Thai National Vaccine Institute (Ministry of Public Health, MOPH) decided to initiate research and development on rabies vaccine to set up an effective vaccine to protect against actively circulating viral strains in it and the neighboring countries. The candidate vaccine aims to provide a local cost-effective production with a potential for international licensure.

The main objective of this study was to select a candidate vaccine rabies virus strain that comply with mandatory characteristics, accordingly to WHO's regulations, including: the ability to efficiently growth in Vero cell line; having an Immunofluorescence companion test using monoclonal anti G protein for antigen detection, and to be one of the most common strain genotype circulating in Thailand. With such objectives online, selected rabies strain genotype sequences were analyzed and characterized by their identity to the rabies virus strain actively circulating in Thailand. The identified Rabies virus TH2 strain is largely endemic throughout Thailand; it was recognized as a potential candidate for the Rabies vaccine development by showing all required and suitable characteristics. Although, the Rabies virus TH2 strain appears genetically distant from the commercially available vaccine strain in Thailand, it should stimulate immunity and prevent most of the rabies risk due to the actively circulating dog rabies strain in Thailand.

Keywords: Fix virus; Local strain; Rabies virus; Seed selection

Introduction

Rabies is an acute viral infection of the nervous system that presents both encephalitic and paralytic clinical forms. Rabies is transmitted by the rabies virus (*Rabies lyssavirus*) through saliva of infected mammal (e.g. dog, cat, and domestic ungulate), rarely the human to human transmission occurs, while exceptionally the virus can be transmitted through organ or tissue transplant [1]. The *Rabies lyssavirus*, is a negative sense, single-stranded enveloped RNA virus of the *Rhabdoviridae* family presenting in a typical cylindrical morphology in electron-micrograph. The genetic information of 11,615 to 11,966 pair base is packaged as a ribonucleoprotein complex tightly bound to the viral nucleoprotein while the genome encodes for five proteins including: nucleoprotein (N), glycoprotein (G), phosphoprotein (P), matrix protein (M), and polymerase (L) [2]. Although rabies is a well-known ancient disease, its virus appears endemic but particularly in low and medium-income countries (LMIC) while human rabies continues to be an important public health problem particularly in Asia and Africa [3]. The combined annual cost for both continents is estimated to be US\$ 583.5 million yearly and mostly due to post exposure prophylaxis [4]. As it's generally observed in Thailand and LMIC, the canine appears as the primary vector of rabies virus [5]. A human case of Rabies was first identified in Thailand in the 1920s and then in canines. While felines and stray animal populations grew, rabies cases also increased with a record high of 370 people death in 1980 [6]. After several outbreaks that occurred in the 1980s, canine and human rabies decreased due to an efficient post-exposure treatments, education, mas, dog and cat vaccination campaign [6]. In 2018, Thailand presented one of its major rabies outbreaks spreading among

14 provinces (Buri ram, Rayong, Songkhla, Surin, Trang, Nakhon Ratchasima, Prachuap Khiri khan, Phatthalung, Nong khai, Yasothon, Klalasin, Mukdahan, Tak, Surat Thani), with a mortality rate of 0.03 per 100,000 inhabitants and 17 fatal cases recorded [7]. In the meanwhile, 1,469 (15.8%) confirmed cases of rabies in animals (mostly dog and cattle) from 54 provinces were reported (Rabies Situation - 7 Oct 19 /2020 - Department of Livestock Development Ministry of Agriculture and Cooperatives). After this recent and unforeseen event, the Thai government decided to put an end to these surprising epidemic outbreaks, the cost of post exposure treatment and animal vaccination remain at a very high cost due to the need to import the anti-rabies vaccine from abroad. Therefore, the National Vaccine Institute (NVI, MOPH, and Thailand) asked the Center for Vaccine Development (Institute of Molecular Biosciences, Mahidol University) and the National Institute of Health (Thailand Department of Medical Science and MOPH) to investigate the potential for the development of a human vaccine using a vaccine strain complying with international regulation. The purpose of this study was to identify and select a rabies virus strain among the entire rabies virus isolated in Thailand and stored at the bio bank of the National Institute of Health (Thai Ministry of Medical Sciences, MOPH). These strains were chosen to exhibit specific and mandatory characteristics as recommended by the World Health Organization [8] and consistently having the ability to replicate in the Vero cell line. After this preliminary phase of identifying with high accuracy a targeted strain, the future development of inactivated human rabies vaccine will be completed under the direction of the MOPH and the Government Pharmaceutical Organization (GPO).

Materials and Methods

Cell lines

Two cell lines commercially available from ATCC were selected and used respectively for virus amplification and virus replication effectiveness, including: a mouse cell line derived from mouse (*Mus musculus*) Neuroblastoma (MNA, ATCC CCL-131); and the Vero cell line derived from normal kidney cells of the adult African green monkey (*Cercopithecus aethiops*) (ATCC CCL-81).

Rabies virus

Fifty Rabies virus strains were provided by Thai National Institute of Health including thirteen strains from human and thirty-seven strains from canine's brain. All the strains tested positive by Indirect Fluorescent Assay (IFA) using the FITC Anti-Rabies Monoclonal Globulin (Product number 800-092)

Indirect immunofluorescent antibody test (IFAT)

IFAT was performed using an anti-nucleoprotein monoclonal antibody, commercially available (FITC Anti-Rabies Monoclonal Globulin, Fujirebio US, Inc) following a classical previously described method [9].

Rabies virus adaptation and selection

The 50 rabies virus strains were propagated in Mouse Neuroblastoma (MNA) and passaged to time on this cell line at 37°C under 5% CO₂. Propagated strains were then selected for their response by IFAT and, only 5 strains only showed a positivity $\geq 2+$ by IFAT (undiluted cell suspension having $\geq 50\%$ of fluorescent cell were considered as

positive). The five selected strains were then propagated in MNA cell line for five or more passages and then used for the virus to be titrated by Reed and Muench method (fluorescence titration) and quantitative evaluated by Nested RT-PCR [10]. Ultimately, the five Rabies virus strains, showing a titer $>10^4$ Tissue Culture Infective Dose (TCID), were selected and propagated into Vero cell line kept at 5% CO₂, 37 °C, and followed by 5 to 10 passages .

Deep sequencing

The RNA extraction was performed on an archived selected TH 13-42-07240 virus strains (i.e. TH2 clusters) sample isolated from canine brain in 2009 [11]. From a Vero cell infected monolayer, i.e. TH 13-42-07240 rabies virus strain tested positive by IFAT, viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Germantown, MD). Total RNA was depleted of host genomic DNA and ribosomal RNA (rRNA) using NEBNext rRNA depletion kit (New England Biolabs, UK) following the manufacturer's instructions, eluting the final product on in 30 ml molecular grade water from the collection, previously de water. The depleted RNA was then purified to remove the enzyme using the RNeasy plus mini kit (QIAGEN, Germantown, MD), without DNase digestion and eluted in 30 ml of molecular grade water. cDNA was synthesized using Maxima H minus Double stranded cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA), following the manufacturer's instructions.

Genotype sequence analysis

Characterizing the viral genome, we used a random amplification, deep-sequencing approach (454 Sequencing) as described elsewhere, where 98% of reads matched the Rhabdovirus genome reference (Accession number EU293111) [12]. The multiple sequence alignment was based on Rhabdovirus genome reference strain 8743THA with the Rabies virus strain TH 13-42-07240 (TH2 clusters) and performed using Clustal Omega v1.2.0 [13] with default parameters. The DNA sequence variations between the Rabies virus strain 13-42-07240 sequences and the reference sequence (EU293111) were analyzed with the DnaSP software (version 5.10.1) to identify synonymous and non-synonymous sites.

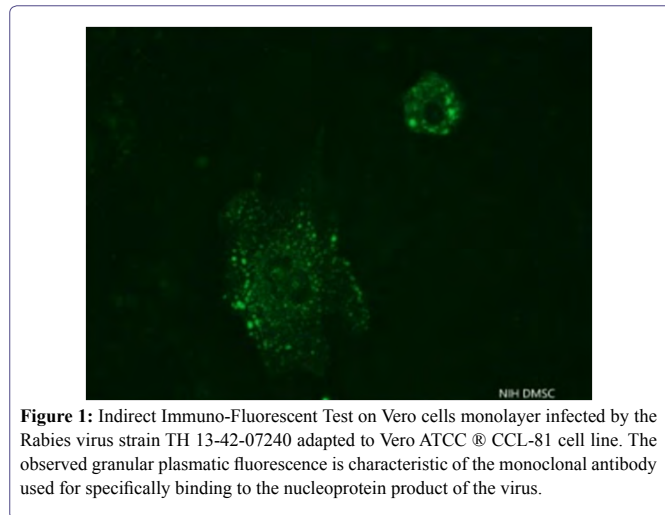
Phylogenetic analysis

The trimmed original sequences were aligned with all Rabies virus sequences available from GenBank (May 2019) using Multiple Alignment of Fast Fourier Transform (MAFFT) [14]. Preliminary phylogenetic analyses were done using PhyML (maximum likelihood), implemented in Seaview Version 4, a multiplatform graphical user interface for sequence alignment and phylogenetic tree building [15]. Main phylogenetic analysis were performed on a subset of sequences, representing the overall genetic diversity of Rabies virus, under a Bayesian statistical framework implemented with BEAST package v.1.8.4 [16] using the model that fits best to the data accordingly with the corrected Bayesian Information Criterion (BICc) obtained in Jmodeltest2® [17].

Results

Following the WHO's recommendation, Rabies vaccine virus strain candidate should have a titer $\geq 6 \log_{10}$ TCID₅₀ (WHO, 2005). From the fifty rabies virus selected strains, after more than five passages in MNA cell and then passaged five to ten times in Vero cell line, only one strain, the Rabies virus strain TH 13-42-07240, was

found positive by IFAT (4+, 100%), 1/50 (2%) and replicates efficiently at first passage in Vero cell (Figure 1) and after the 6th passages, it showed a titer of 6 log₁₀ TCID₅₀ on day 4 (MOI 0.1) and 6.2 log₁₀ TCID₅₀ on day 3 (MOI 0.01) (Table 1).



Day	Rabies virus strain TH 13-42-07240	
Post-Infection	MOI 0.1	MOI 0.01
0	3*	2.1
1	3.6	2.8
2	4.6	4.3
3	5.6	6.2
4	6	5.6
5	5.8	5.4
6	5.5	5.3
7	5	5

Table 1: Complete genome sequences of rabies virus used for genetic analysis.

Altogether, 251 strain sequences were used for phylogenetic analysis of the G1 nucleoprotein gene and compared to the rabies virus strain TH 13-42-07240 (Figure 1).

Also, the rabies virus strain TH 13-42-07240 was different from the commonly use Rabies virus strain 13-42-07240 vaccine (Table 2, Figure 2), the TH 13-42-07240 rabies virus strain isolated from a canine brain appears widely distributed throughout Thailand (Table S1).

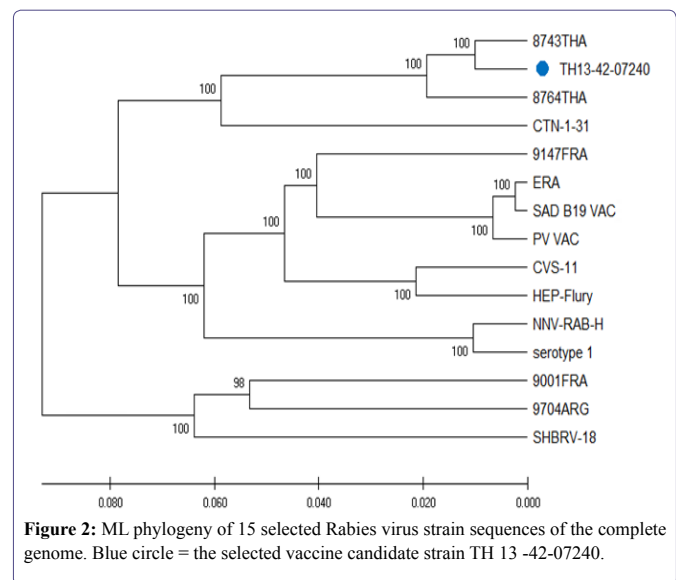
Discussion

Our study, with respect to the animal, use for Rabies virus amplification for diagnosis appears clearly unnecessary when for the first time NMA cell line were used to efficiently amplify Rabies virus followed by its adaptation to Vero cell culture. With respect to the TH 13-42-07240 rabies virus strain characteristics, a Rabies virus strain comply with WHO recommendation (i.e Vero cell line optimal titer, and availability of the companion IFA test) appears to be a strong candidate for the development of inactivated rabies vaccine that

should efficiently stimulated immunity to prevent rabies epidemic in Thailand [21,22]. Furthermore, the rabies virus strain TH 13-42-07240 appears to actively circulate in Thailand and China and has the potential to be used for Rabies vaccine development of interest for the region [18]. Although, it has some genetic distance with the other commercial vaccine in the market, as a local strain isolated in Thailand it has been proven to efficiently by neutralizing circulated rabies virus strain [19,20].

Gen Bank Acc. no.	Reference no.	Species	Origin	Year
EU293121	8743THA	Human	Thailand	1983
EU293111	8764THA	Human	Thailand	1983
EU293115	9147FRA	Fox	France	1991
EU293113	9001FRA	Dog	French Guyana	1990
EU293116	9704ARG	Bat	Argentina	1997
AY705373	SHBRV-18	Bat	USA	1983
EF437215	NNV-RAB-H	Human	India	2006
EF206709	SAD B19	Vaccine	-	-
NC_001542	PV	Vaccine	France	1882
HQ317918	CTN-1-31	Human	China	1956
GQ918139	CVS-11	-	France	1882
EF206707	ERA	Vaccine	-	-
AB085828	HEP-Flury	Vaccine	USA	1939
AY956319	Serotype 1	Saliva	Germany	-
MN075931	TH13-42-07240	Dog	Thailand	1989

Table 2: Complete genome sequences of rabies virus used for genetic analysis.



Conclusion

Rabies virus strain TH 13-42-07240 originally isolated from Thailand, comply with international recommendation for Rabies candidate vaccine development, it is one of the most abundant strains in the South Eastern Asian peninsula, and therefore appears as a preferred candidate for Rabies vaccine development in Thailand.

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Supplementary Material

	Isolation no.	Species	Origin	Year
AY849022	5NBm	Dog	Nonthaburi	2001
AY849023	26NPpmt	Dog	Nakhon Pathom	2001
AY849024	53SPppd	Dog	Samut Prakan	1999
AY849025	67PTtyb	Dog	Pathum Thani	1999
AY849026	80PTilk	Dog	Pathum Thani	1999
AY849027	81NBtm	Dog	Nonthaburi	2001
AY849028	99Mkl	Dog	Pathum Thani	1999
AY849029	79PTm	Dog	Pathum Thani	1999
AY849030	125SSktb	Dog	Samut Sakhon	1999
AY849031	133SSm	Dog	Samut Sakhon	1999
AY849032	176SSktb	Dog	Samut Sakhon	1999
AY849033	187SSm	Dog	Samut Sakhon	1999
AY849034	207SSm	Dog	Samut Sakhon	1999
AY849035	217SSm	Dog	Samut Sakhon	1999
AY849036	218NBbk	Dog	Nonthaburi	2001
AY849037	222NBbbt	Dog	Nonthaburi	1999
AY849038	228NBtm	Dog	Nonthaburi	1999
AY849039	304NPbl	Dog	Nakhon Pathom	2001
AY849040	315NPm	Dog	Nakhon Pathom	2001
AY849041	324NPm	Dog	Nakhon Pathom	2001
AY849042	91PTns	Dog	Pathum Thani	1999
AY849043	95NBtm	Dog	Nonthaburi	1999
AY849044	108PTilk	Dog	Pathum Thani	1999
AY849045	112PTtyb	Dog	Pathum Thani	1999
AY849046	195NBby	Dog	Nonthaburi	1999
AY849047	215NPncs	Dog	Nakhon Pathom	1999
AY849048	232NPsp	Dog	Nakhon Pathom	1999
AY849049	235NBpk	Dog	Nonthaburi	1999
AY849050	263NPm	Dog	Nakhon Pathom	1999
AY849051	C267BKbkn	Cat	Bangkok	2000
AY849052	C269PTm	Cat	Pathum Thani	2000
AY849053	C271BKrtv	Cat	Bangkok	2001
AY849054	C274BKdd	Cat	Bangkok	1999
AY849055	C276BKpv	Cat	Bangkok	1999
AY849056	C277BKkt	Cat	Bangkok	1999
AY849057	303KJtmk	Dog	Kanchanaburi	2001
AY849058	308KJm	Dog	Kanchanaburi	2001
AY849059	318KJtmk	Dog	Kanchanaburi	2001
AY849060	326KJtm	Dog	Kanchanaburi	2001
AY849061	333KJm	Dog	Kanchanaburi	2001
AY849062	335KJpnt	Dog	Kanchanaburi	2001
AY849063	89PTib	Dog	Pathum Thani	1999
AY849064	38/43	Dog	Chaiyaphum	2000
AY849065	39/43	Dog	Chaiyaphum	2000
AY849066	46/43	Dog	Chaiyaphum	2000
AY849067	23SPppd	Dog	Samut Prakan	2001
AY849068	33/43	Dog	Chaiyaphum	2000
AY849069	34/43	Dog	Chaiyaphum	2000
AY849070	19/43	Dog	Chaiyaphum	2000
AY849071	51BKds	Dog	Bangkok	1999
AY849072	62SPbp	Dog	Samut Prakan	1999

AY849073	48BKpyt	Dog	Bangkok	2001
AY849074	HM65BK	Human	Bangkok	1998
AY849075	HM75BK	Human	Bangkok	1998
AY849076	HM88BKjj	Human	Bangkok	1999
AY849077	HM208BKpv	Human	Bangkok	2001
AY849078	404PLkcs	Cattle	Phatthalung	2002
AY849079	355UThk	Dog	Uthai Thani	2001
AY849080	415PLtm	Dog	Phatthalung	2002
AY849081	294CPm	Dog	Chaiyaphum	2002
AY849082	295CPksb3	Dog	Chaiyaphum	2002
AY849083	351NSm	Dog	Nakhon Sawan	2001
AY849084	353CNmnr	Dog	Chai Nat	2001
	363NScs	Dog	Nakhon Sawan	2001
AY849086	412SKsd	Dog	Songkla	2002
AY849087	414SKm	Dog	Songkla	2002
AY849088	361NSly	Dog	Nakhon Sawan	2001
AY849089	376SHib	Dog	Sing Buri	2001
AY849090	380UTbr	Dog	Uthai Thani	2001
AY849091	393LBm	Dog	Lop Buri	2001
AY849092	384SHib	Dog	Sing Buri	2001
AY849093	411NTht	Dog	Nakhon Si Thammarat	2002
AY849094	374CNspy	Dog	Chai Nat	2001
AY849095	362CNm	Dog	Chai Nat	2001
AY849096	357UTm	Dog	Uthai Thani	2001
AY849097	356UTth	Dog	Uthai Thani	2001
AY849098	381NStk	Dog	Nakhon Sawan	2001
AY849099	408SKhy	Dog	Songkla	2002
AY849100	332SBm	Dog	Suphan Buri	2001
AY849101	354CNhk	Dog	Chai Nat	2001
AY849102	425PN	Dog	Phisanulok	2002
AY849103	389LBm	Dog	Lop Buri	2001
AY849104	423PN	Dog	Phisanulok	2002
AY849105	424PN	Dog	Phisanulok	2002
AY849106	413SKhy	Cattle	Songkla	2001
AY849107	358CNsbr	Dog	Chai Nat	2001
AY849108	317SBspn	Dog	Suphan Buri	2001
AY849109	334PJsry	Dog	Prachuap Khiri Khan	2001
AY849110	340SBspn	Dog	Suphan Buri	2001
AY849111	400SKm	Dog	Songkla	2001
AY849112	87BKsl	Dog	Bangkok	2001
AY849113	156PTns	Dog	Pathum Thani	1999
AY849114	182CCbnp	Dog	Chachoengsao	1999
AY849115	349PBm	Dog	Phetchaburi	2001
AY849116	307RBptr	Dog	Ratchaburi	2001
AY849117	329PBm	Dog	Phetchaburi	2001
AY849118	406STm	Dog	Satun	2002
AY849119	352NStk	Dog	Nakhon Sawan	2001
AY849120	301RBm	Dog	Ratchaburi	2001
AY849121	157PJsry	Dog	Prachuap Khiri Khan	1999
AY849122	237NYm	Dog	Nakhon Nayok	1999
AY849123	270ATvsc	Cat	Ang Thong	2001
AY849124	281NRm	Dog	Nakhon Ratchasima	2001
AY849125	282NRpc	Dog	Nakhon Ratchasima	2001
AY849126	305RBbp	Dog	Ratchaburi	2001

AY849127	306RBptr	Dog	Ratchaburi	2001
AY849128	191AYsn	Dog	Ayuthaya	1999
AY849129	151SBdc	Dog	Suphan Buri	1999
AY849130	22CBkkm	Dog	Chanthaburi	2001
AY849131	162PCm	Dog	Phichit	1999
AY849132	319PBm	Dog	Phetchaburi	2001
AY849133	86SPm	Dog	Samut Prakan	2001
AY849134	136CLblm	Dog	Chon Buri	1999
AY849135	396YLM	Dog	Yala	2001
AY849136	250AYbt	Dog	Ayuthaya	1999
AY849137	302RBpt	Dog	Ratchaburi	2001
AY849138	316SMm	Dog	Samut Songkhram	2001
AY849139	426PN	Dog	Phisanulok	2002
AY849140	296CPksb	Dog	Chaiyaphum	2002
AY849141	313PBnyp	Dog	Phetchaburi	2001
AY849142	288NRbl	Dog	Nakhon Ratchasima	2002
AY849143	505KBlT	Dog	Krabi	2000
AY849144	507TRrd	Dog	Trang	2000
AY849145	511NTis	Dog	Nakhon Si Thammarat	2000
AY849146	515KBlT	Dog	Krabi	2000
AY849147	524KBkn	Dog	Krabi	2000
AY849148	559TRpl	Cattle	Trang	2000
AY849149	578KBkt	Dog	Krabi	2001
AY849150	584TRkt	Dog	Satun	2001
AY849151	603KBlT	Dog	Krabi	2001
AY849152	595Ylbns	Dog	Yala	2001
AY849153	493RNm	Dog	Ranong	2000
AY849154	656PLppy	Dog	Phatthalung	2002
AY849155	676KSm	Squirrel	Kalasin	2002
AY849156	513PLm	Dog	Phatthalung	2000
AY849157	589YLM	Dog	Yala	2001
AY849158	608SRm	Dog	Satun	2001
AY849159	HMS152S	Human	Si Sa Ket	2001
AY849160	599TRhy	Dog	Trang	2001
AY849161	473BRpk	Dog	Surin	2001
AY849162	485BRpk	Dog	Buri Ram	2002
AY849163	510NTm	Dog	Nakhon Si Thammarat	2000
AY849164	486UMhsp	Dog	Amnat Chareon	2002
AY849165	487Umm	Dog	Amnat Charoen	2002
AY849166	501RNM	Dog	Nakhon Si Thammarat	2002
AY849167	283NRpm	Dog	Nakhon Ratchasima	2001
AY849168	459KSm	Dog	Kalasin	2002
AY849169	463SRsn	Dog	Surin	2001
AY849170	464SRskp	Dog	Surin	2001
AY849171	465SRm	Dog	Surin	2001
AY849172	466SRrbr	Dog	Surin	2001
AY849173	528NTRpb	Dog	Nakhon Si Thammarat	2000
AY849174	548NTlSk	Dog	Nakhon Si Thammarat	2000
AY849175	289NRht	Dog	Nakhon Ratchasima	2001
AY849176	723KKm	Dog	Khon Kaen	2001
AY849177	481BRhr	Dog	Buri Ram	2002
AY849178	494RNm	Dog	Ranong	2000
AY849179	503SNws	Dog	Surat Thani	2002
AY849180	553STdkl	Dog	Khuan Ka Long/Satun	2000

AY849181	666STm	Cattle	Satun	2002
AY849182	458Lam	Dog	RoiEt	2001
AY849183	500SNks	Dog	Surat Thani	2002
AY849184	454Lam	Dog	Roi Et	2001
AY849185	495RNm	Dog	Ranong	2001
AY849186	460Lam	Dog	Roi Et	2002
AY849187	488Umm	Dog	Amnat Charoen	1998
AY849188	489SEkh	Dog	Si Sa Ket	2000
AY849190	502SNcb	Dog	Surat Thani	2002
AY849189	472BRm	Dog	Buri Ram	2001
AY849191	499SNps	Dog	Surat Thani	2002
AY849192	HMS241CL	Human	Chon Buri	2001
AY849193	694LAsp	Dog	Roi Et	2002
AY849194	690NKsps	Dog	Nong Khai	2002
AY849195	689Umm	Dog	Amnat Charoen	2001
AY849196	688Umm	Dog	Amnat Charoen	2002
AY849197	691NKbk	Dog	Nong Khai	2002
AY849198	695Lack	Dog	Roi Et	2002
AY849199	698KSm	Squirrel	Kalasin	2002
AY849200	700KStkt	Dog	Kalasin	2003
AY849201	708YSm	Dog	Yasothon	2000
AY849202	568YLM	Dog	Yala	2000
AY849203	725MDm	Dog	Mukdahan	2001
AY849204	726UBbt	Dog	Ubol Ratchathani	2001
AY849205	738KKm	Dog	Khon Kaen	2002
AY849206	742MDm	Dog	Mukdahan	2002
AY849207	740MDdt	Dog	Mukdahan	2002
AY849208	711SLm	Dog	Sakon Nakhon	2000
AY849209	709SEktr	Dog	Si Sa Ket	2000
AY849210	678cBRm	Cat	Buri Ram	2000
AY849211	728UBm	Cattle	Ubol Ratchathani	2001
AY849212	685BRppe	Dog	Buri Ram	2002
AY849213	714YSm	Dog	Yasothon	2001
AY849214	715YSm	Water buffalo	Yasothon	2001
AY849215	718YSlkk	Dog	Yasothon	2001
AY849216	705MDm	Dog	Mukdahan	2000
AY849217	731UBvrc	Dog	Ubol	2001
AY849218	704KKcp	Dog	Khon Kaen	2000
AY849219	703KKm	Dog	Khon Kaen	2000
AY849220	732KSm	Cattle	Kalasin	2001
AY849221	741YSm	Dog	Yasothon	2002
AY849222	744UBdud	Dog	Ubol Ratchathani	2002
AY849223	713KKcp	Dog	Khon Kaen	2001
AY849224	717UBm	Dog	Ubol Ratchathani	2001
AY849225	707LYm	Dog	Loei	2000
AY849226	131SPpsj	Dog	Samut Prakan	1999
AY849227	HMS223RY	Human	Rayong	2002
AY849228	747PGtp	Dog	Phangnga	2003
AY849229	766PRm	Dog	Phetchabun	2003
AY849230	769PRcd	Dog	Phetchabun	2003
AY849232	774NNm	Dog	Nan	2002
AY849231	773CMcp	Dog	Chiang Mai	2002
AY849233	775PYp	Dog	Phayao	2003

AY849234	776NSTk	Dog	Nakhon Sawan	2002
AY849235	795NSCs	Dog	Nakhon Sawan	2003
AY849236	801SUsk	Dog	Sukhothai	2002
AY849237	802KPIkb	Dog	Kamphaeng Phet	2002
AY849238	811SUkrm	Dog	Sukhothai	2002
AY849239	806PNnm	Dog	Phisanulok	2002
AY849240	814UDm	Dog	Uttaradit	2002
AY849241	807UDts	Dog	Uttaradit	2002
AY849242	800Udm	Dog	Uttaradit	2002
AY849243	815PRIs	Dog	Phetchabun	2002
AY849244	805PCbmn	Dog	Phichit	2002
AY849245	780NSm	Dog	Nakhon Sawan	2003
AY849246	762PRm	Dog	Phetchabun	2003
AY849247	813PNbrk	Dog	Phisanulok	2002
AY849248	796PNbrk	Dog	Phisanulok	2003
AY849249	816PRcd	Dog	Phetchabun	2002
AY849250	778LBtv	Dog	Lop Buri	2003
AY849251	794SUssr	Dog	Sukhothai	2003
AY849252	784Sum	Dog	Sukhothai	2003
AY849253	785SUkkl	Dog	Sukhothai	2003
AY849254	788SUsk	Dog	Sukhothai	2003
AY849255	808SUssn	Dog	Sukhothai	2002
AY849256	793SUsn	Dog	Sukhothai	2003
AY849257	777LBm	Dog	Lop Buri	2003
AY849258	779NSly	Dog	Nakhon Sawan	2003
AY849259	804UDm	Dog	Uttaradit	2002
AY849260	338PJm	Dog	Prachuap Khiri Khan	2001
HQ317918	CTN-1-31	Human	China	1956
EU293115	9147FRA	Fox	France	1991
EU293113	9001FRA	Dog	French Guyana	1990
AY705373	SHBRV-18	Bat	USA	1983
EU293116	9704ARG	Bat	Argentina	1997
EF206709	SAD B19	Vaccine	-	-
EF206707	ERA	Vaccine	-	-
NC_001542	PV	Vaccine	France	1882
AB085828	HEP-Flury	Vaccine	USA	1939
GQ918139	CVS-11	-	France	1882
EF437215	NNV-RAB-H	Human	India	2006
AY956319	Serotype 1	Saliva	Germany	-
MN075931	TH13-42-07240	Dog	Thailand	1989

Table S1: Nucleoprotein Gene sequences of the selected rabies virus strains used for genetic .

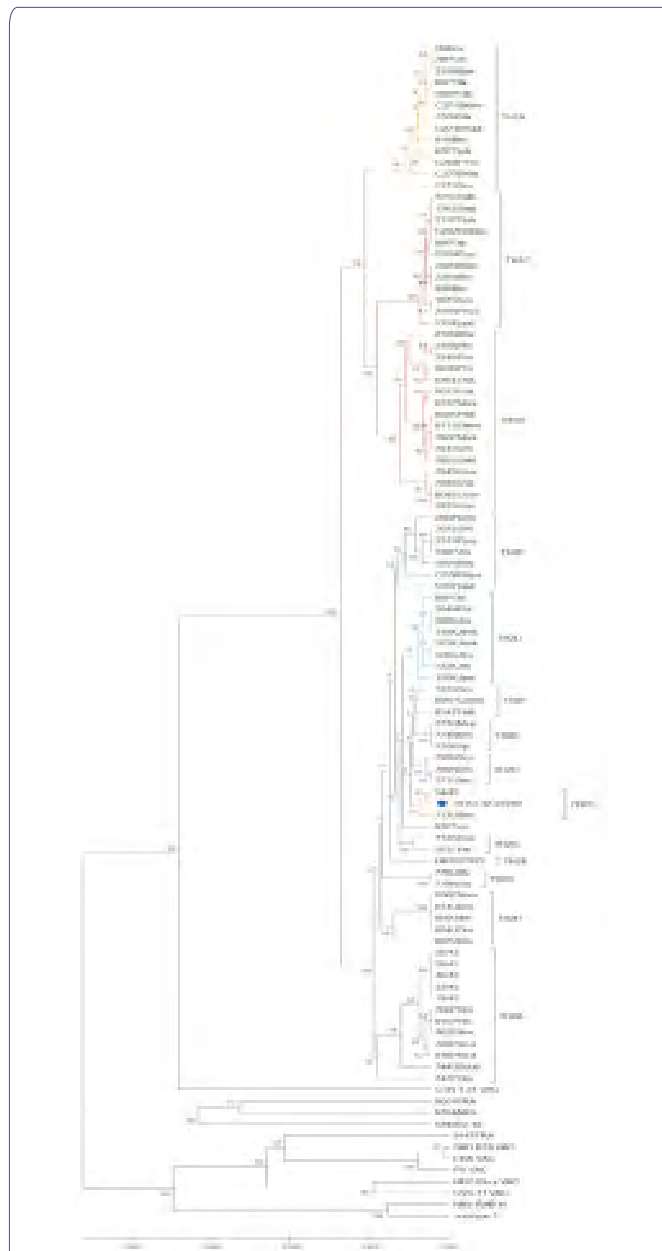


Figure S1: ML phylogeny of xxx sequences from the N-coding region of Rabies virus strains.

Caption: Sequences were obtained from Gen Bank and the TH strain sequenced for this study.



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