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Evaluation and Phylogenetic Analysis of Regular Rabies Virus Vaccine Strains

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Abstract

Background: This study aimed to evaluate Rabies virus vaccine strains. The obtained results may be helpful for vaccine producers and researchers to compare the strains with wild type and other vaccine strains and select the correct strain to challenge their products.

Methods: Fourteen rabies virus vaccine strains were compared with each other. The full genomes of the selected strains were taken from the GenBank and the N, P and G genes were labeled. The major and minor antigenic sites of these sequences were identified and contrasted with each other. The identity matrix was designed for rabies virus full genome, N and G genes. In addition, the phylogenetic tree was drawn based on rabies virus N gene for deep analysis.

Results: Although there were no significant differences between antigenic sites in N, P, and G genes, there were noticeable differences for full genome identity matrix and this significant difference can also be observed in N and G identity matrix. In the phylogenetic tree, the Iranian sequences were distant from currently applied vaccine strains.

Conclusion: It is necessary to pay attention to the results shown in phylogenetic tree because they warn us about distance between the Iranian sequences and current strains used in applied vaccines. In addition, the obtained results help vaccine producers to choose a correct strain to challenge their product and evaluate their vaccine potency.

Keywords: Identity matrix, Phylogenetic tree, Rabies virus, Vaccine strains

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Introduction

Rabies virus is one of the most fatal viruses which causes death in humans and other mammals unless regular treatments are administrated properly.[1] This virus is a member of *Lyssavirus* genus from Rhabdoviridae family,[2] and is a rod- or bullet-like, single-stranded RNA, negative-sense, non-segmented and enveloped virus.[3,4] The genomic length of rabies virus is about 12 kb and encodes 5 proteins consisting of nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and a viral RNA polymerase (L).[2,5,6] Ribonucleoprotein (RNP) complex is composed of the nucleoprotein, phosphoprotein and polymerase (4). The matrix and the glycoprotein are involved in virus assembling and budding.[7] The glycoprotein also acts as virus receptor and helps virus penetrate into the target cells.[4] There are seven recognized genotypes (GT) of lyssaviruses including rabies virus (RABV, GT1, and the classical rabies disease agent), Lagos bat virus (LBV, GT2), Mokola virus (MOKV, GT3), Duvenhage virus (DUVV,

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GT4), European bat lyssavirus types 1 and 2 (EBLV-1, GT5 and EBLV-2, GT6, respectively) and Australian bat lyssavirus (ABLV, GT7). All genotypes except MOKV have bat reservoirs.[8]

Rabies infection transmits to the human through the bite of rabid animals such as dogs, raccoons, skunks, wolves, foxes and bats.[2] Although infection commonly occurs by a bite, the transmission may also happen via eating infected carcasses.[9] This virus causes over 60 000 human deaths every year all around the world.[10] The major animals that play a role in human rabies infection are different from continent to continent. Most of the human rabies infections and deaths mainly occur in developing Asian and African countries.[11] Most of the provinces in Iran are infected with rabies virus, but most infections happen in north, northwest and northeast regions.[12]

Vaccination plays an important role in controlling this zoonotic disease.[13,14] By this method, neutralizing antibodies provide the most effective adaptive immune response to control the infection, which are produced against the RNP, and especially the G antigen.[14–16]

There are numbers of rabies vaccine strains used for anti-rabies vaccine development, including RV-97, RC-HL, Ni-Ce, Nishigahara (Nishi), Pittman-Moore (PM), SAD B19, SRV9, Evelyn Rokitniki Abelseth (ERA), Pasteur virus (PV), HEP-Flury, PM1503, LEP-Flury and NNV-RAB-H (NNV).[17–19]

The glycoprotein of rabies virus is the most dominant antigen which has multiple major and minor antigenic sites. Major antigenic site I harbors both conformational and linear epitopes and is situated at position 226–231 of mature glycoprotein. Major antigenic site II involves two stretches in position 34–42 and 198–200, major antigenic site III is a continuous conformational epitope at residues 330–338, and epitope IV with one amino acid is located at position 251. Minor antigenic site "a" or G1 is at position 342-343,[20-25] and G5 is positioned between 261–264 residues as another minor antigenic site. The amino acid at position 333 of the mature glycoprotein is found to be associated with viral pathogenicity.[25-27] Antigenic sites I and IV, and antigenic sites II and III on the N protein are composed of linear and conformational dependent epitopes, respectively.[28] Antigenic sites I and IV are mapped to a region composed of 24 amino acid residues in the C terminal part of the N protein.[29]All three epitopes of antigenic site I and two epitopes of site IV are positioned on 358-367 residues, and the other epitope of site IV is mapped at 375-383 residues.[30] In P protein, one antigenic site is mapped at position 191–206,[31] and two more epitopes are located in position 75–90.[32]

In this study, the major and minor antigenic sites of glycoprotein (G) in addition to the nucleoprotein (N) and the phosphoprotein (P) were compared to each other. The full genomes, N and G genes identity matrix were drawn and the exact distance between strains was determined. In addition, the N gene of vaccine strains and N gene of wild type strains from most regions of the world were analyzed by phylogenetic analysis and evaluation. The results of this study can be helpful for phylogenetic comparison of the regular vaccine strains with each other, and also with wild type strains. Besides, this study may be helpful for vaccine producers and researchers to compare the strains with wild type and other vaccine strains and even select the correct strain to challenge their products.

Materials and Methods

Dataset

The full genomes data set contained PV-2061 (JX276550.1), PV (M13215-PMID: 3459163-3407152), CVS-11 (GQ918139-PMID: 23858717), CVS-N2c (HM535790-PMID: 21068252), PM1503 (DQ099525), Lep-Flury (DQ099524), Hep-Flury (AB085828-PMID: 12505638), RC-HL (AB009663-PMID: 11270607), SADB19 (M31046-PMID: 2139267), SRV9 (AF499686), ERA (EF206707-PMID: 18485548), RV-97 (EF542830-PMID: 18187223), Ni-Ce (AB128149-PMID: 17010466), Nishgahara (AB044824-PMID: 11270607). SHBRV-18 (AY705373-PMID: 15520387). These data sets were used as out group.

Comparison of Vaccine Strains Antigenic Sites

The antigenic and the immune-dominant sites of the G, N and P genes were determined. Their sequences were translated and the antigenic sites were compared with each other in a chart.

Vaccine Full Genomes, G and N Genes Identity Matrix

For exact comprehensive view, the full genomes, N and G genes identity matrix from above strains were drawn by BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and the exact distance between them was determined.

Phylogenetic Analysis of Vaccine Strains Versus the Wild Type Strains

The N gene of the wild type strains from Iran and most of the regions of the world were taken from the GenBank and analyzed phylogenetically with N and G genes of the vaccine strains. Nucleotide sequences were aligned using CLUSTAL W software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Phylogenetic tree was drawn using maximum likelihood methods (1000 times bootstrap sampling) with molecular evolutionary genetics analysis

(MEGA) software version 6.6. The Kimura 2-parameter model was used with a transition/transversion ratio of 1.5 and statistical support of the phylogenetic tree structure was obtained with 1000 bootstrap replicates. Significance was based on bootstrap values of 75%.

Results

Comparison of Vaccine Strains Antigenic Sites

Antigenic and immune-dominant sites of the G, N and P genes from selected strains were compared with each other. As expected, most of the variations and substitutions in amino acids occurred in selected antigenic sites of the SHBRV-18 strain. In regular vaccine strains for evaluation of G, there are no substitutions in antigenic sites I, IV and G1. In antigenic site IIa of the RC-HL strain, Valine was replaced with Alanine, but in site IIb, CVS-11, CVS-N2c, PM1503 and Lep-Flury strains have Glutamate instead of Glycine. In antigenic site III, the Hep-Flury strain has glutamine instead of arginine, and in the srv9 strain, serine and valine are replaced with arginine and isoleucine, respectively. In this position, the Lep-Flury and RV-97 strains contain Histidine instead of Arginine. Also, in the RC-HL, Ni-Ce and Nishgahara strains, Phenylalanine is replaced with leucine in G5 antigenic site. All of the above strains have Arginine in position 333 except Hep-Flury and SRV9 strains. The full results of the comparison are shown in Table 1.

Full Genomes, N and G Genes Identity Matrix

The identity matrixes of Regular rabies virus vaccine strains are shown in Tables 2 and 3. These tables depict the maximum and minimum differences between the strains. For instance, the maximum and minimum identities in full genomes are specified between PV-2061 strain with M13215-PV and HEP–Flury strains, respectively.

Phylogenetic Analysis of Vaccine Strains Versus Wild Type Strains

The N genes of the wild type strains are clustered in different clads and genotypes in the phylogenetic tree including Cosmopolitan, Arctic-like and Arctic, Africa 2 and 3, India and Asia. The N and G genes sequences of the vesicular stomatitis virus (Indiana subtype) were used as out-group. Figure 1 shows the distance between clads where the regular vaccine strains were just positioned in cosmopolitan clad. The obtained results indicate the distance between vaccine and other genotype 1 wild type strains.

Discussion

In the first week after anti-rabies vaccination, the host can mount active immune response. This immunity is directly against the rabies virus (RV) glycoprotein (G) and mediates the complement lysis and/or antibody-dependent cellular cytotoxicity of RV-infected cells.[33] This protection is not solely dependent on the levels of virus-neutralizing antibodies induced by the G protein. N protein, as the major constituent of the internal RNP complex and also as the most conserved protein, has an important role in induction of neutralizing antibodies.[34] The P protein potentially represents a useful alternative antigen for lyssavirus discrimination.[35] Moreover, it can be recognized by both Class I and Class II restricted T Cells.[31] It is a common belief that G and N proteins are considered to be the most important proteins for immunogenicity,[19] and in lyssavirus genotyping N gene divergence is the standard consideration.[36]

In this study, 14 rabies virus vaccine strains were contrasted with each other. SHBRV strain, the farthest candidate of genotype 1, was used as control throughout the analysis. So, the full genomes of the selected strains were taken from gene bank and the N, P and G genes were labeled. The selected genes were aligned and translated to first structure of protein sequence. As shown in Table 1, the major and minor antigenic sites of these sequences were identified and compared with each other.

The RNA-dependent RNA polymerase (L protein) of the rabies virus does not have proof-reading mechanism.[37,38] In each geographical region of the world, the survival of the virus population is dependent upon a successful balance between virus adaptations, evolution and escape of the host immunity system. The rabies virus L protein provides this ability for the virus. All proteins of rabies virus are essential for virus life cycle and are related to each other. This functional and structural relationship forces virus to develop co-evolution of its own proteins simultaneously,[39] and lyssavirus genes probably have the same value for phylogenetic analyses.[36]

Although there were no significant differences between antigenic sites in N, P, and G genes, there were noticeable differences for full genome identity matrix and this significant difference can also be seen in N and G identity matrix (Tables 2 to 4).

Each approved strain has its own origin and is able to produce maximum protection in related area; so, there are several rabies virus vaccine strains which can be used for development of anti-rabies vaccine.[17-19] Genetic knowledge of the vaccine strains would provide the significant compatibility between the vaccine strains and the wild type viruses circulating in different regions. For example, to obtain maximum protection in some adjacent area such as south-eastern Finland and north-western Russia, two different vaccine strains are in use.[19]

Iran is one of the highly endemic regions for rabies virus. During 2002–2011, there were 1 188 579 cases of treatment with post exposure prophylaxis (PEP) in Iran.

Table 1. Vaccine Strains Antigenic Site Comparison

Protein	Strains	Antigenic Sites						
		I	IIa	IIb	III	IV	a(G1)	G5
		226–231	198–200	34–42	330–338	251	342–343	261–264
G	pv-2061	KLCGVL	KRA	GCTNLSGFS	KSVRTWNEI	w	KG	HDFR
	M13215-PV	KLCGVL	KRA	GCTNLSGFS	KSVRTWNEI	w	KG	HDFR
	CVS-11	KLCGVL	KRA	GCTNLS**E**FS	KSVRTWNEI	w	KG	HDFR
	CVS-N2c	KLCGVL	KRA	GCTNLS**E**FS	KSVRTWNEI	w	KG	HDFR
	PM1503	KLCGVL	KRA	GCTNLS**E**FS	KSVRTWNEI	w	KG	HDFR
	Lep-Flury	KLCGVL	KRA	GCTNLS**E**FS	KSVRTWNEI	w	KG	HDF**H**
	Hep-Flury	KLCGVL	KRA	GCTNLSGFS	KSV**Q**TWNEI	w	KG	HDFR
	RC-HL	KLCGVL	KR**V**	GCTNLSGFS	KSVRTWNEI	w	KG	HD**L**R
	SADB19	KLCGVL	KRA	GCTNLSGFS	KSVRTWNEI	w	KG	HDFR
	SRV9	KLCGVL	KRA	ECTNLSGFS	KSV**S**TWNE**V**	w	KG	HDFR
	ERA	KLCGVL	KRA	GCTNLSGFS	KSVRTWNEI	w	KG	HDFR
	RV-97	KLCGVL	KRA	GCTNLSGFS	KSVRTWNEI	w	KG	HDF**H**
	Ni-Ce	KLCGVL	KRA	GCTNLSGFS	KSVRTWNEI	w	KG	HD**L**R
	Nishgahara	KLCGVL	KRA	GCTNLSGFS	KSVRTWNEI	w	KG	HD**L**R
	SHBRV-18	KLCGV**P**	K**K**A	GCT**S**LSGFS	KSVRTWNE**V**	w	KG	HDF**H**

Protein	Strains	Antigenic sites		
		I	IV	
		358–367	359–366	375–383
N	pv-2061	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	M13215-PV	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	CVS-11	RFFRDEKELQ	FFRDEKEL	TK**S**DVALAD
	CVS-N2c	RFFRDEKELQ	FFRDEKEL	TK**S**DVALAD
	PM1503	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	Lep-Flury	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	Hep-Flury	RFFRDEKELQ	FFRDEKEL	TKTDVALAD

Protein	Strains	Antigenic Sites		
		I	IV	
		358–367	359–366	375–383
N	RC-HL	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	SADB19	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	SRV9	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	ERA	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	RV-97	RFFRDE**R**ELQ	FFRDE**R**EL	TKTDVALAD
	Ni-Ce	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	Nishgahara	RFFRDEKELQ	FFRDEKEL	TKTDVALAD
	SHBRV-18	RFFRDEKELQ	FFRDEKEL	TKT**EM**ALAD

Protein	Strains	Antigenic Sites	
		75–90	191–206
P	pv-2061	GKYREDFQMDEGEDPS	EEDDLSVEAEIAHQIA
	M13215-PV	GKYREDFQMDEGEDPS	EEDDLSVEAEIAHQIA
	CVS-11	GKYREDFQMDEGEDP**N**	EEDDLSVEAEIAHQIA
	CVS-N2c	GKYREDFQMDEGEDP**N**	EEDDLSVEAEIAHQIA
	PM1503	GKYREDFQM**G**EGEDP**N**	EEDDLSVEAEIAHQIA
	Lep-Flury	GKYREDFQMDEGEDP**N**	EEDDLSVEAEIAHQIA
	Hep-Flury	GKYREDFQM**N**EGEDP**N**	**G**EDDLSVEAEIAHQA
	RC-HL	G**RHQ**EDFQMDEGEDPS	EEDDLSVEAEIAHQIA
	SADB19	GKYREDFQMDEGEDPS	EEDDLSVEAEIAHQIA
	SRV9	GKYREDFQMDEGEDPS	EEDDLSVEAEIAHQIA
	ERA	GKYREDFQMDEGEDPS	E**K**DDLSVEAEIAHQIA
	RV-97	GKYREDFQMDEGEDPS	EEDDLS**A**EAEI**T**HQIA
	Ni-Ce	G**RHQ**ED**P**QMDEGEDPS	EEDDLSVEAEIAHQIA
	Nishgahara	G**RHQ**EDFQMDEGEDPS	EEDDLSVEAEIAHQIA
	SHBRV-18	**S**K**CQ**EDFQMDE**A**EDP**A**	EEDD**P**SVEAEIAHQA

Table 2. Regular Rabies Virus Vaccine Strains Identity Matrix (Full Genome)

Strains	pv-2061	M13215-PV	CVS-11	CVS-N2c	PM1503	Lep-Flury	Hep-Flury	RC-HL	SADB19	SRV9	ERA	RV-97	Ni-Ce	Nishgahara	SHBRV-18
pv-2061	ID	0.998	0.901	0.901	0.887	0.893	0.884	0.902	0.984	0.984	0.988	0.907	0.906	0.907	0.808
M13215-PV	0.998	ID	0.9	0.901	0.886	0.892	0.884	0.902	0.985	0.984	0.988	0.907	0.905	0.907	0.808
CVS-11	0.901	0.9	ID	0.999	0.977	0.943	0.932	0.891	0.9	0.9	0.902	0.897	0.894	0.896	0.809
CVS-N2c	0.901	0.901	0.999	ID	0.977	0.942	0.931	0.891	0.901	0.9	0.902	0.897	0.895	0.896	0.81
PM1503	0.887	0.886	0.977	0.977	ID	0.958	0.915	0.876	0.886	0.886	0.888	0.883	0.88	0.882	0.796
Lep-Flury	0.893	0.892	0.943	0.942	0.958	ID	0.945	0.881	0.892	0.891	0.893	0.885	0.884	0.886	0.797
Hep-Flury	0.884	0.884	0.932	0.931	0.915	0.945	ID	0.872	0.884	0.883	0.885	0.876	0.875	0.877	0.792
RC-HL	0.902	0.902	0.891	0.891	0.876	0.881	0.872	ID	0.902	0.902	0.903	0.926	0.987	0.989	0.804
SADB19	0.984	0.985	0.9	0.901	0.886	0.892	0.884	0.902	ID	0.999	0.994	0.906	0.904	0.906	0.808
SRV9	0.984	0.984	0.9	0.9	0.886	0.891	0.883	0.902	0.999	ID	0.994	0.906	0.904	0.906	0.808
ERA	0.988	0.988	0.902	0.902	0.888	0.893	0.885	0.903	0.994	0.994	ID	0.908	0.906	0.908	0.809
RV-97	0.907	0.907	0.897	0.897	0.883	0.885	0.876	0.926	0.906	0.906	0.908	ID	0.93	0.932	0.805
Ni-Ce	0.906	0.905	0.894	0.895	0.88	0.884	0.875	0.987	0.904	0.904	0.906	0.93	ID	0.997	0.806
Nishgahara	0.907	0.907	0.896	0.896	0.882	0.886	0.877	0.989	0.906	0.906	0.908	0.932	0.997	ID	0.808
SHBRV-18	0.808	0.808	0.809	0.81	0.796	0.797	0.792	0.804	0.808	0.808	0.809	0.805	0.806	0.808	ID

Table 3. Regular Rabies Virus Vaccine Strains Identity Matrix (N Gene)

Strains	pv-2061	M13215-PV	CVS-11	CVS-N2c	PM1503	Lep-Flury	Hep-Flury	RC-HL	SADB19	SRV9	ERA	RV-97	Ni-Ce	Nishgahara	SHBRV-18
pv-2061	ID	0.998	0.893	0.895	0.895	0.906	0.906	0.9	0.978	0.975	0.981	0.902	0.906	0.908	0.798
M13215-PV	0.998	ID	0.895	0.896	0.896	0.906	0.906	0.902	0.98	0.977	0.983	0.903	0.907	0.909	0.798
CVS-11	0.893	0.895	ID	0.998	0.996	0.943	0.942	0.881	0.892	0.89	0.893	0.886	0.885	0.887	0.798
CVS-N2c	0.895	0.896	0.998	ID	0.994	0.944	0.944	0.882	0.893	0.891	0.895	0.887	0.886	0.888	0.799
PM1503	0.895	0.896	0.996	0.994	ID	0.944	0.944	0.881	0.893	0.891	0.895	0.886	0.886	0.888	0.797
Lep-Flury	0.906	0.906	0.943	0.944	0.944	ID	0.984	0.886	0.902	0.9	0.904	0.888	0.891	0.893	0.806
Hep-Flury	0.906	0.906	0.942	0.944	0.944	0.984	ID	0.883	0.902	0.9	0.904	0.883	0.888	0.89	0.805
RC-HL	0.9	0.902	0.881	0.882	0.881	0.886	0.883	ID	0.901	0.899	0.902	0.913	0.984	0.985	0.793
SADB19	0.978	0.98	0.892	0.893	0.893	0.902	0.902	0.901	ID	0.997	0.996	0.904	0.906	0.907	0.799
SRV9	0.975	0.977	0.89	0.891	0.891	0.9	0.9	0.899	0.997	ID	0.994	0.902	0.904	0.906	0.798
ERA	0.981	0.983	0.893	0.895	0.895	0.904	0.904	0.902	0.996	0.994	ID	0.906	0.907	0.909	0.8
RV-97	0.902	0.903	0.886	0.887	0.886	0.888	0.883	0.913	0.904	0.902	0.906	ID	0.92	0.921	0.794
Ni-Ce	0.906	0.907	0.885	0.886	0.886	0.891	0.888	0.984	0.906	0.904	0.907	0.92	ID	0.997	0.796
Nishgahara	0.908	0.909	0.887	0.888	0.888	0.893	0.89	0.985	0.907	0.906	0.909	0.921	0.997	ID	0.797
SHBRV-18	0.798	0.798	0.798	0.799	0.797	0.806	0.805	0.793	0.799	0.798	0.8	0.794	0.796	0.797	ID

Table 4. Regular Rabies Virus Vaccine Strains Identity Matrix (G Gene)

Strains	pv-2061	M13215-PV	CVS-11	CVS-N2c	PM1503	Lep-Flury	Hep-Flury	RC-HL	SADB19	SRV9	ERA	RV-97	Ni-Ce	Nishgahara	SHBRV-18
pv-2061	ID	0.998	0.893	0.895	0.895	0.906	0.906	0.9	0.978	0.975	0.981	0.902	0.906	0.908	0.798
M13215-PV	0.998	ID	0.895	0.896	0.896	0.906	0.906	0.902	0.98	0.977	0.983	0.903	0.907	0.909	0.798
CVS-11	0.893	0.895	ID	0.998	0.996	0.943	0.942	0.881	0.892	0.89	0.893	0.886	0.885	0.887	0.798
CVS-N2c	0.895	0.896	0.998	ID	0.994	0.944	0.944	0.882	0.893	0.891	0.895	0.887	0.886	0.888	0.799
PM1503	0.895	0.896	0.996	0.994	ID	0.944	0.944	0.881	0.893	0.891	0.895	0.886	0.886	0.888	0.797
Lep-Flury	0.906	0.906	0.943	0.944	0.944	ID	0.984	0.886	0.902	0.9	0.904	0.888	0.891	0.893	0.806
Hep-Flury	0.906	0.906	0.942	0.944	0.944	0.984	ID	0.883	0.902	0.9	0.904	0.883	0.888	0.89	0.805
RC-HL	0.9	0.902	0.881	0.882	0.881	0.886	0.883	ID	0.901	0.899	0.902	0.913	0.984	0.985	0.793
SADB19	0.978	0.98	0.892	0.893	0.893	0.902	0.902	0.901	ID	0.997	0.996	0.904	0.906	0.907	0.799
SRV9	0.975	0.977	0.89	0.891	0.891	0.9	0.9	0.899	0.997	ID	0.994	0.902	0.904	0.906	0.798
ERA	0.981	0.983	0.893	0.895	0.895	0.904	0.904	0.902	0.996	0.994	ID	0.906	0.907	0.909	0.8
RV-97	0.902	0.903	0.886	0.887	0.886	0.888	0.883	0.913	0.904	0.902	0.906	ID	0.92	0.921	0.794
Ni-Ce	0.906	0.907	0.885	0.886	0.886	0.891	0.888	0.984	0.906	0.904	0.907	0.92	ID	0.997	0.796
Nishgahara	0.908	0.909	0.887	0.888	0.888	0.893	0.89	0.985	0.907	0.906	0.909	0.921	0.997	ID	0.797
SHBRV-18	0.798	0.798	0.798	0.799	0.797	0.806	0.805	0.793	0.799	0.798	0.8	0.794	0.796	0.797	ID

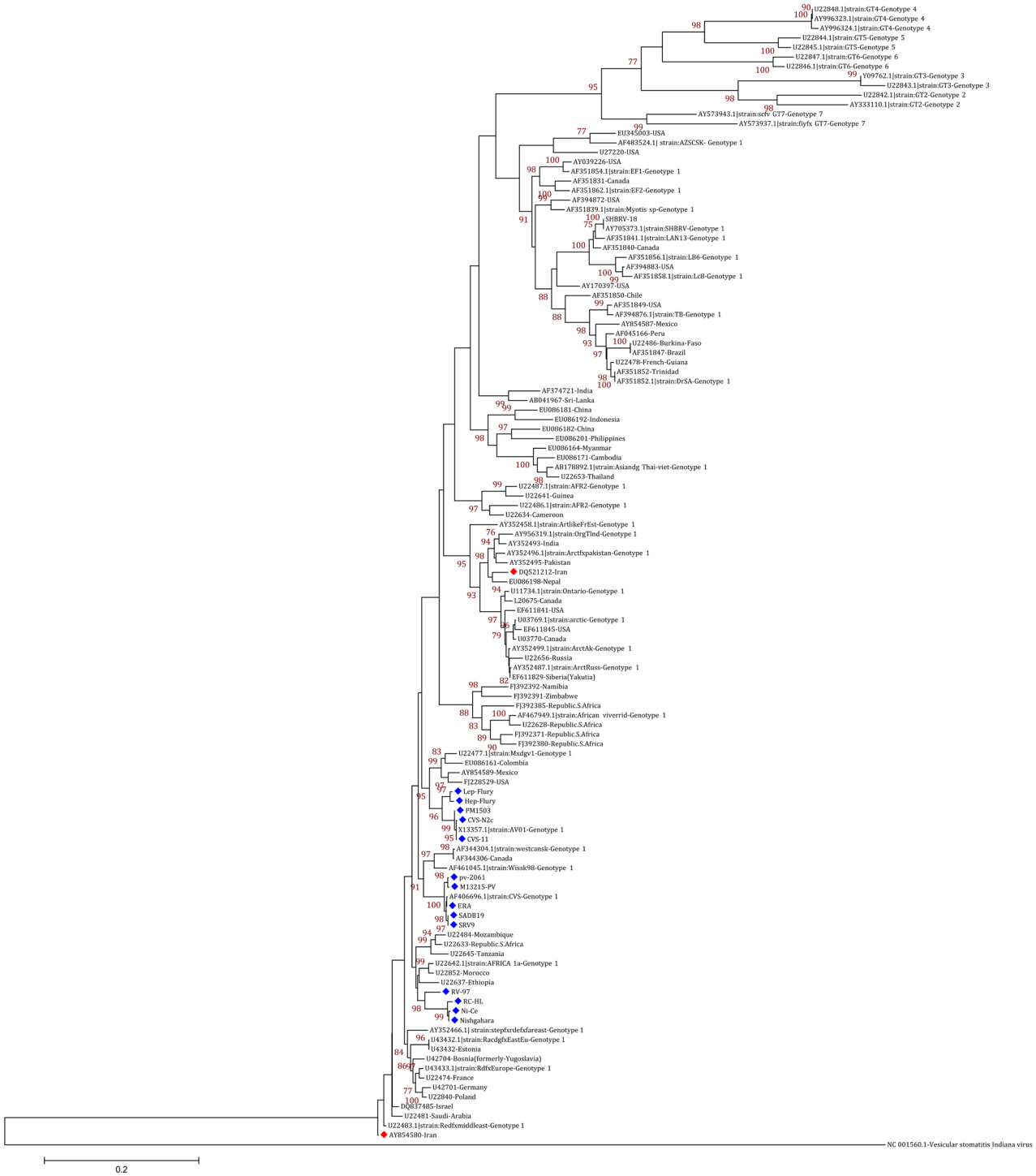

Figure 1. Maximum Likelihood of Wild Type Strains Versus Vaccine Strains N Gene Phylogenetic Analysis. All of the vaccine strains are located in Cosmopolitan cluster. N gene sequence of the vesicular stomatitis virus (Indiana subtype) was used as an out-group. Bootstrap sampling is 1000 that is shown on the trees.

In spite of proper vaccination program, 16 human deaths were associated with rabies. In addition to necessity of extending rabies awareness programs and more accurate vaccine administration,[40] the authorities must pay more attention to choosing the correct vaccine strain for administration in Iran. At the moment, all exposed individuals receive VERORAB from Sanofi-Pasteur or Rabipur from Novartis, India. The strains used in these vaccines are Pittman-Moore (PM), and LEP-Flury, respectively. It is believed that reverse genetics technology will be used to modify different strains of virus to produce the next generation of rabies vaccines to induce more immunity by only a single dose of vaccination.[13] Ajorloo et al constructed rabies virus minigenome which

is a feasible system to evaluate desired modifications in full genome to establish a new generation of vaccine.[41]

In the current study, the phylogenetic tree indicates that sequences of Iranian viruses have distance from Pittman-Moore (PM) and LEP-Flury strains in applied vaccines. One of the Iranian N genes is positioned in Cosmopolitan clade and the other one is located in Arctic like and Arctic clade. None of the Iranian N genes have close relation with Pittman-Moore (PM), or LEP-Flury. The authorities must pay attention to the results shown in phylogenetic tree because it warns us about the distance between the Iranian sequences and current strains in the applied vaccines. The G tree also represents similar results.

The obtained results not only warn authorities to do extensive phylogenetic analysis in the Iranian wild type strains to select and develop a standard vaccine strain, but also help vaccine producers to choose the correct strain for their product challenge and evaluating their vaccine potency.

For the following study, we recommend to select more Iranian sequences and do the phylogenetic analysis. We also recommend a survey to find a consensus reference virus to produce a national strain for vaccine production either inside Iran or abroad.

Authors' Contribution

MA, HM, MN; Data analysis, writing of the manuscript. AA; writing of the manuscript. YS, SS, KS; Systematic search and data extraction. NT, PD, MAR, FSM; Systematic search, sequence management. HRJ, MM, HRN; literature review and data extraction.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

This study is a secondary analysis and does not require an ethical statement.

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