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Short communication

Genetic analysis of the matrix and non-structural genes of equine influenza virus (H3N8) from epizootic of 2008–2009 in India

Nitin Virmani, B.C. Bera, K. Shanumugasundaram, B.K. Singh, B.R. Gulati, R.K. Singh *, R.K. Vaid

National Research Centre on Equines, Sirsa Road, Hisar 125 001, Haryana, India

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ABSTRACT

India faced an epizootic of equine influenza in 2008–2009. The isolated viruses were typed as H3N8 and grouped with the clade 2 viruses of Florida sublineage on the basis of haemagglutinin (HA) gene sequence analysis. This report describes the genetic analysis and selection pressure of matrix (M) and non-structural 1 (NS1) genes of the Indian isolates. All isolates shared 98.41% and 99.54% homology with other clade 2 viruses of Asian origin for M1 and M2 amino acid (aa) sequences, respectively. There were 3 and 4 unique aa residue changes respectively in M1 and M2 proteins in all Asian isolates. Phylogenetic analysis revealed clustering of Indian and Chinese isolates in a separate group designated here as Asian clade for M gene. Indian and Chinese isolates shared homology ranging from 98.17% to 99.08% at aa level. The M and NS1 genes were under negative selection pressure with estimated magnitude of pressure (ω) 0.054, 0.581 and 0.30 for M1, M2 and NS1, respectively.

1. Introduction

Equine influenza (EI) is caused by influenza A viruses of family *Orthomyxoviridae*. Influenza in equines is caused by two types of virus, H3N8 and H7N7 however, EI due to H7N7 has not been reported for more than three decades from any part of the world. Since the first isolation of H3N8 virus (Miami/63), these viruses have diversified into various lineages *viz.* Predivergent, Eurasian and American – which has further diversified into Kentucky, South America and Florida sublineage (clade 1 & 2) (Bryant et al., 2009; Lai et al., 2001; Daly et al., 1996). In recent times (2007 onwards), Florida clade 1 viruses have been implicated in outbreaks in Australia and Japan (Callinan, 2008; Yamanaka et al., 2008) while clade 2 viruses have been responsible for the outbreaks in Asian countries including Mongolia (2007), China (2007–2008) and India (2008–2009) (Virmani et al., 2010; Qi et al., 2010).

Haemagglutinin (HA) and neuraminidase (NA) genes encode the major surface proteins and are considered highly variable due to constant host immune pressure (Obenauer et al., 2006). The internal protein coding genes such as M and NS are relatively under low selection pressure, however, they have earlier been shown to evolve in parallel to the HA gene (Lindstrom et al., 1998). Detailed functional and evolutionary studies of these genes have been carried out in influenza viruses of human, avian and swine species (Hale et al., 2008a,b; Gomez-Puertas et al., 2000; Hughey et al., 1995). These studies indicate that mutational changes/reassortment in the M and NS genes may have serious implications on the pathogenesis and virulence of the influenza viruses. Matrix gene encodes M1 and M2 proteins which are responsible for determining host range, virus assembly and budding of viral particles (Gomez-Puertas et al., 2000; Naffakh et al., 2008), while NS1 gene is a determinant of virulence of influenza viruses and suppresses the host immune response (Wang et al., 2000; Hale et al., 2008a). It also helps in regulation of synthesis of viral RNA, splicing of mRNA and translation process (Wang and Krug, 1998; Burgui et al., 2003; Hale et al., 2008a). While no studies are available in respect of

* Corresponding author. Tel.: +91 1662 275787; fax: +91 1662276217.
E-mail address: rks.nrce@gmail.com (R.K. Singh).

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EIVs for the recent changes in the M genes and their evolution, the reassortment has been observed in the NS1 gene of EIVs from clade1 of Florida sublineage which have the sequence from EIVs of older Eurasian and American lineage (Bryant et al., 2009). This paper describes the genetic analysis and selection pressure of M and NS1 genes of EIV isolates from 2008 to 2009 epizootic in India.

2. Materials and methods

2.1. Viruses

H3N8 equine influenza viruses (EIVs) from epizootic (2008–2009) of EI in India were propagated in embryonated hen's eggs at third passage level. The EIVs used for the present study were A/eq/Jammu- Katra/06/08, A/eq/Mysore/12/08, and A/eq/Gopeshwar/1/09.

2.2. RT-PCR and sequencing of M and NS genes

Viral RNA was isolated from 200 μl allantoic fluid of infected embryos using AuPrep Viral RNA extraction kit (Life Technologies (India) Pvt. Ltd., India) according to manufacturer's protocol. The purified RNA was reverse transcribed into cDNA as described previously (Virmani et al., 2010). The resultant cDNA was used in subsequent PCR amplifications using gene-specific primers (forward: 5′-ATGGATTCCAACACTGTGTCAA-3′ and reverse: 5′-TTTCTCGTTTCAGCTTATTTAA-3′ for NS gene; forward: 5′-ATGAGTCTTCTGACCGA GG-3′ and reverse: 5′-TTACTC-CAGCTCTATGT TGAC-3′ for M gene). The amplified products were cloned into pTZ57R/T vector (MBI Fermentas, Burlington, Canada) and sequenced. Nucleotide sequences were submitted to GenBank and assigned

Table 1
Amino acid substitutions in the predicted M1 and M2 sequence compared to A/eq/Katra-Jammu/6/08.

Strain	M1																
	14	15	30	35	50	80	85	91	95	101	142	188	208	224	231	248	253
Equine/KAT/06/08	I	I	N	K	P	I	S	N	K	R	V	A	K	S	D	M	*
Equine/MYS/12/08	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/GOP/1/09	.	.	D	N	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/CZECH/09		V	D	.	.	V	.	.	R	.	.	.	R	.	.	.	*
Equine/GAN/7/08	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/XIN/1/07	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/MON/8/08	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	L	*
Equine/NEW/5/03	.	V	D	.	.	V	.	.	R	.	.	.	.	.	.	.	*
Equine/OHI/1/03	.	V	D	.	.	V	.	.	R	.	.	.	R	.	.	.	*
Equine/KEN/5/02	.	V	D	.	.	V	.	.	R	.	.	.	.	.	.	.	*
Equine/NEW/1/93	V	V	D	.	.	V	.	.	R	.	.	.	.	.	.	.	*
Equine/NEW/2/93	.	V	D	.	.	V	.	.	R	.	.	.	.	.	.	.	*
Equine/FON/1/79	.	V	D	.	.	V	.	.	R	.	.	T	.	.	.	.	*
Equine/MIA/1/63	.	V	D	.	.	V	N	.	R	.	.	.	Q	.	N	.	*
Chicken/LAOS/07	.	V	D	.	.	V	N	.	R	K	.	.	Q	.	N	.	*
Duck/BEIJ/61/05	.	V	D	.	.	V	N	.	R	.	.	.	Q	.	.	.	*
Duck/VICT/92	.	V	D	.	.	V	N	.	R	K	.	.	Q	.	.	.	*
Canine/MIAM/05	.	V	D	.	.	V	.	.	R	.	.	.	R	.	.	.	*
Canine/FLO/03	.	V	D	.	.	V	.	.	R	.	.	.	R	.	.	.	*

Strain	M2																			
	17	18	21	23	27	33	43	48	50	59	61	69	70	74	82	83	85	87	89	98
Equine/KAT/06/08	C	K	G	S	V	I	L	S	F	L	R	P	E	E	N	A	S	D	S	*
Equine/MYS/12/08	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/GOP/1/09	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/CZECH/09	.	.	D	.	.	.	.	F	.	M	.	.	.	.	.	.	D	.	G	*
Equine/GAN/7/08	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/XIN/1/07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/MON/8/08	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*
Equine/NEW/5/03	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	.	G	*
Equine/OHI/1/03	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	E	G	*
Equine/KEN/5/02	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	.	G	*
Equine/NEW/1/93	.	.	D	.	.	.	.	F	.	.	.	R	.	D	.	.	D	.	G	*
Equine/NEW/2/93	.	.	.	.	.	V	.	F	.	.	.	.	.	.	.	.	D	.	G	*
Equine/FON/1/79	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	.	G	*
Equine/MIA/1/63	.	.	D	.	.	.	.	F	C	.	.	.	.	.	S	.	D	.	G	*
Chicken/LAOS/07	.	.	D	.	.	.	.	F	C	.	.	.	.	.	S	.	D	.	G	*
Duck/BEIJ/61/05	.	.	D	.	.	.	.	F	C	.	.	.	.	.	S	.	D	.	G	*
Duck/VICT/92	.	.	D	.	I	.	I	F	C	.	.	.	.	.	S	.	D	.	G	*
Canine/MIAM/05	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	.	G	*
Canine/FLO/03	.	.	D	.	.	.	.	F	.	.	.	.	.	.	.	.	D	.	G	*

Amino acid residues are numbered from the N-terminal methionine. Residue identity to A/eq/Katra-Jammu/06/08 is shown with a dot (.) and stop codons are represented with an asterisk (*). Other reference strains are A/eq/Mysore/12/08, A/eq/Gopeshwar/1/09, A/eq/Czech-Republic/09, A/eq/Gansu/7/08, A/eq/Hubei/6/08, A/eq/Xinjiang/1/07, A/eq/Inner Mongolia/8/08, A/eq/Newmarket/5/03, A/eq/Ohio/1/03, A/eq/Kentucku/5/02, A/eq/Newmarket/1/93, A/eq/Newmarket/2/93, A/eq/Fontainebleau/1/79, A/eq/Miami/1/63, A/chicken/Laos/07, A/duck/Beijing/40/05, A/duck/Victoria/92, A/canine/Miami/05, A/canine/Florida/242/03.