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Differentiation of Marek's Disease Virus Isolates from India by Sequence Analysis of Meq Gene

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ABSTRACT

The main aim of the present study was to investigate sequence diversity and mutations in the *Meq* gene of Marek's disease viruses (MDV) isolated in India. To understand the molecular characteristics of MDV *Meq* gene, the sequence generated from Directorate of Poultry Research (DPR) farm and commercial poultry farms isolates were compared with published sequences of Indian isolates. Sequence analysis showed that all of the isolates contained an open reading frame (ORF) of 1020 nucleotides, which encoded a 339 amino acid peptides. In the present phylogenetic study, *Meq* gene sequence generated from MDV strains of DPR isolate was found to share a clade with previously published DPR strains depicting homogenecity in MDV strain prevalent in DPR farm of Hyderabad. The *Meq* gene sequence of MDV strains amplified from commercial poultry farm found to be evolutionary closer with Ludhiana isolates. Amino acid sequence decoded from *Meq* gene sequence revealed that four consecutive proline repeats are three in number in DPR isolates while it ranges from 4-5 quadruple proline repeats in commercial farms samples. The published MDV strains from India has 4-5 consecutive four proline repeats in strains from the commercial farms. In the present study, a single point mutation was recorded in *Meq* gene sequence of DPR isolate where proline was replaced by serine at 233 positions. This mutation is recorded for the first time in Meq gene sequence of Indian isolates. The present study shows that the MDV strains from DPR farm were more virulence than other Indian isolates.

Keywords: Marek's disease virus, Meq gene, Sequence analysis

Marek's disease virus is taxonomically placed in the subfamily *Alpha herpesvirinae* and genus *Mardivirus*. The Mardiviruses includes *Gallid herpesvirus 2* (also known as Marek's Disease Virus type 1 or MDV), *Gallid herpesvirus 3* (Marek's Disease Virus type 2 or GaHV3) and *Meleagrid herpesvirus 1* (Herpesvirus of Turkeys or HVT) (Davison *et al*., 2009). The issue of major concern is the evolution of MDV towards greater virulence with concomitant reduction of vaccine efficacy, high mortality, expansion of host range (quails, turkeys) and crossing the age resistance barrier (Witter, 1996). On the basis of the ability to induce MD lesions in vaccinated chickens, serotype 1 MDV strains have been grouped as mild (mMDV), virulent (vMDV), very virulent (vvMDV) and very virulent plus (vv+MDV) pathotypes (Witter, 1997). At present, most of the viruses isolated from problem flocks in the field are typed as vvMDV or vv+MDV (Davison and Nair, 2005; Zhang *et al*., 2011). Isolation and culture of MDV field strains are essential for monitoring changes in the predominant strains, and to evaluate the effectiveness of existing vaccines (Tan *et al*., 2008).

Meq is a 339-amino acid long protein encoded within the MDV EcoRI Q fragment of serotype 1 (Jones *et al*., 1992). There are two copies of *Meq* in the MDV genome, one in each of the repeat long regions (TRL and IRL) (Lee *et al*., 2008). It is consistently expressed in all MDV tumor and latent cells and is only present in serotype 1 strains but not in non-oncogenic serotypes 2 and 3 of MDV (Lee *et al*., 2008). Among the MD viral determinants of oncogenicity, the basic leucine zipper protein *Meq* is considered to be the most important and the most extensively studied. Deleting the *Meq* proteins or abolishing some of the

important interactions does affect the oncogenicity of the virus (Kung *et al*., 2001; Nair, 2013). Now it is widely accepted that the *Meq* gene is one of the most important genes involved in the determination of virulence of an MDV isolate (Lee *et al*., 2008; Lupiani *et al*., 2004).

In India, there are many reports discussing the isolation and epidemiology of MDV circulating in relation to vaccine failure in MDV-vaccinated broiler, layer and breeder flocks (Arulmozhi *et al*., 2011). However, so far, there are no available data about the molecular composition of MDV strains circulating in India, and their phylogenetic relationship with each other and with other strains in the database. Here we provide the first report comparing sequences for *Meq* gene of MDV strains prevalent in India. The goal of our research was to identify mutations in genes encoded by MDVs of distinctly different virulence levels that correlate with those distinct pathotypes. This study could provide useful information as to the nature of MDVs circulating in India.

MATERIALS AND METHODS

Experimental design

Twenty-six and ten samples were collected from Directorate of Poultry Research (DPR) farms and commercial poultry farm located nearby Hyderabad respectively. From each bird, the feather follicle, nerve, spleen, and tumour were collected aseptically and stored at -20^{o}C for molecular detection of MDV by PCR.

Detection of MDV by PCR and sequence analysis

Frozen tissues samples of feather follicle, nerve, spleen, and tumour were homogenized individually under sterile conditions with B.P blade on Petri dish. The DNA extracted from tissue homogenate by standard Phenol: Chloroform: Isoamyl Alcohol method (Sambrook and Russell, 2001). In the present study, the published PCR primers of *Meq* gene were used for the identification of *Gallid herpesvirus 2* (Burgess, 2003). PCR products were separated on 1.5% (w/v) Agarose (HiMedia Laboratories Pvt. Ltd.) gel containing 0.5 µg/ml Ethidium bromide (Sambrook and Russell, 2001).

The amplified gene was outsourced to Amnion Biosciences, Bengaluru for double-stranded sequencing and the sequence generated was analyzed using DNAStar software. To screen for mutations in the *Meq* gene, the forward and reverse sequence data were aligned, analyzed and compared using EditSeq, MegAline software (DNAStar Inc., Madison) and MEGA version 6.0 respectively. The *Meq* gene sequences of MDV strains from DPR and commercial poultry farms were submitted to GeneBank with accession number KX619428 and KT795530, respectively. The sequences were compared with the published isolates retrieved from GenBank (Table 1).

Table 1: Details of the MDV published sequence used in the present study for phylogenetic analysis

Sl. No.	Isolate name	Gene	Place	Host	Accession no.
1	DPR *Meq* 1	*Meq*	Telangana (CPF)	Chicken	KT795529
2	DPR *Meq* 2	*Meq*	Telangana (CPF)	Chicken	KT795530
3	DPR *Meq* 3	*Meq*	Telangana (DPRF)	Chicken	KT795531
4	DPR *Meq* 4	*Meq*	Telangana (DPRF)	Chicken	KX619428
5	LDH-3262	*Meq*	Ludhiana	Chicken	KF895035
6	LDH-2929	*Meq*	Ludhiana	Chicken	KF895034
7	LDH-2700	*Meq*	Ludhiana	Chicken	KF895033
8	LDH-2614	*Meq*	Ludhiana	Chicken	KF895032
9	LDH-2483	*Meq*	Ludhiana	Chicken	KF895031
10	LDH-2003	*Meq*	Ludhiana	Chicken	KF895030
11	LDH-1758	*Meq*	Ludhiana	Chicken	KF895029
12	tn-n3	*Meq*	Tamil Nadu	Chicken	HM749326
13	tn-n1	*Meq*	Tamil Nadu	Chicken	HM749324
14	tn-n2	*Meq*	Tamil Nadu	Chicken	HM749325

RESULTS AND DISCUSSION

Full-length sequencing of *Meq* gene revealed 1198 bp product (Fig. 1), however, 1020 bp were subjected to phylogenetic analysis with other published Indian strains which are found to group into three distinct clades (Fig. 2).

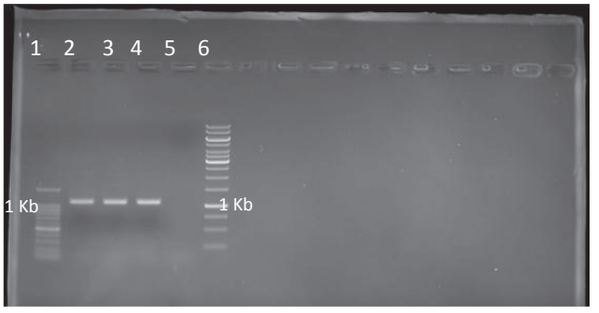

Fig. 1: PCR amplification products of MDV1 using MEQF and MEQR primer pairs

Lane 1	:	100bp ladder
Lane 2	:	DPR *Meq* 4 (DPRF) sample
Lane 3	:	DPR *Meq* 2 (CPF) sample
Lane 4	:	Positive control
Lane 5	:	Negative control
Lane 6	:	1kb ladder

Meq gene from MDV strains of DPR was found to share the clade with previously published DPR strains. The *Meq* gene sequence of MDV strains from commercial poultry farm showed an evolutionary relationship with Ludhiana strains. Tamil Nadu strains form a separate clade with high bootstrap value indicating evolutionary distinctness from other Indian strains. Four consecutive proline repeats were observed in MDV strains from DPR farm, however, it ranges from 3-5 in other published strains from India. In the present study, a single point mutation was evidenced at 233 positions wherein proline is replaced by serine. The comparison of *Meq* sequences of MDV strains from DPR and published Indian MDV strains revealed 98.5 to 100% similarity.

The strains of DPR are evolutionary distinct from commercial poultry farms in and around Hyderabad. The MDV strains from commercial poultry farms showed a closer relationship with strains from Ludhiana. This observation indicates that the strains from DPR and commercial poultry farms were different even though these were collected from the same region. Amino acid sequence decoded from *Meq* gene sequence revealed that four consecutive proline repeats are three in number in DPR isolates while it ranges from 4-5 quadruple proline

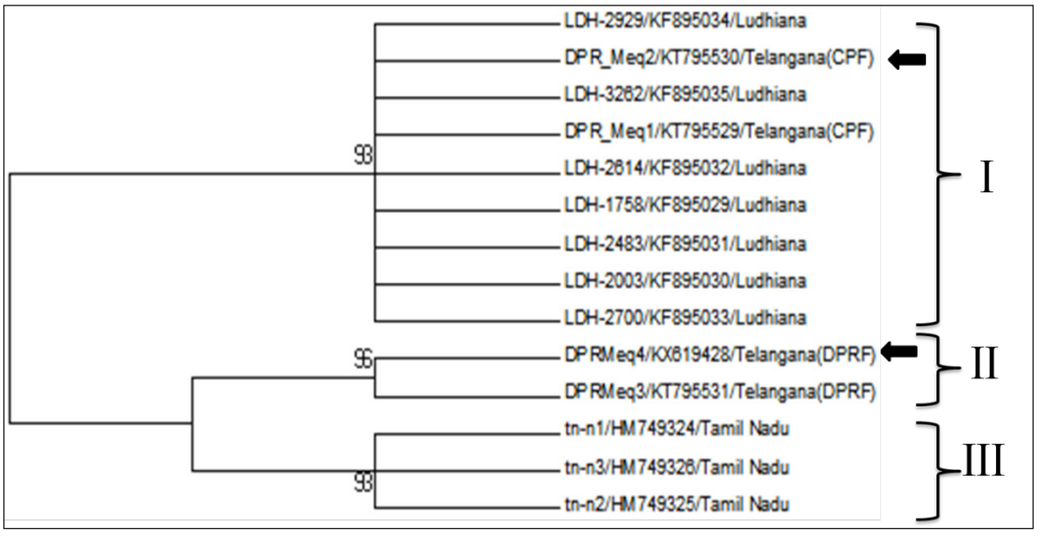

Fig. 2: Phylogenetic analysis of DPR *Meq* 2 and DPR *Meq* 4 (Arrow) strains with MDV published Indian strains available in the NCBI database –*Meq* gene sequences by the Neighbor-joining method using Tamura-Nei statistical model (Bootstrap samples n=1000; Cut off 75).

repeats in commercial farms samples. The three proline repeats (PPPP) are reported to be strongly associated with MDV virulence (Renz *et al.,* 2012). The *Meq* gene of DPR stains showed point mutation of serine by proline at the 233 position. The present study concluded the presence of new and more virulent strains of MDV circulating in the birds of DPR farms.

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