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Detection of oncogenic strains of MDV serotype 1 in Tamil Nadu by duplex PCR

KURUNCHI C DIVYA[1], M PARTHIBAN[2], G SATHISH [3], K KUMANAN[4] and B MOHAN[5]

Tamil Nadu Animal and Veterinary Sciences University, Chennai, Tamil Nadu 600 007 India

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ABSTRACT

The present study was to increase the diagnostic sensitivity of PCR, duplex PCR for detection of *Meq* gene, and *132 bp repeat* sequence of Marek's disease virus (MDV) serotype 1 was used. Out of 60 samples collected from different suspected areas in Tamil Nadu, 8 samples showed the presence of amplified products of 1.06 kb *(Meq* gene) and 434bp *(tandem repeats*), whereas no amplification was observed in a virulent vaccine strain (serotype 3). Sequence homology of *meq* gene of these strains were confirmed with published sequences. These results clearly indicated that the detection of oncogenic strains of MDV serotype 1 in Tamil Nadu poultry populations.

Key words: Marek's disease viruses, *Meq* gene, Polymerase Chain Reaction, 132 bp repeat sequence

In India, Marek's disease was observed in 1970, the time when poultry industry experienced a sudden spurt, both because of import of foreign breeds and also due to intensive rearing (Devprakash and Rajya 1970). Marek's disease virus from different parts of India were reported namely Punjab, Haryana, Delhi, Tamil Nadu, Asom, Andhra Pradesh, Karnataka and Gujarat. Sridhar *et al*. (1997) reported the mortality due to Marek's disease and avian leukosis complex in commercial layer farms at Namakkal, Tamil Nadu. Recently very virulent serotype 1 of MDV was reported in Tamil Nadu by Raja *et al.* (2009).

The Marek's disease virus *BamHI-H* region received attention as a pathogenic determinant following demonstration of the expansion of the *132-bp tandem repeats* within the *BamHI-D/BamHI-H* region during attenuation (Peng *et al.* 1992). All current research on Marek's disease indicated that MDV continues to evolve strains of greater virulence. Shamblin *et al.* (2004) analyzed strains of MDV of different levels of virulence to determine if mutations in a particular gene were responsible for the increase in virulence of the virus. However, it was found that the *meq* gene contained polymorphisms and point mutations that seemed to directly correlate with MDV virulence.

Becker *et al.* (1992) used MDV-1 specific primers for *meq, 132bp repeats, pp38* did not amplify MDV-2 (SB-1) and MDV-3 (HVT) DNA. Sung *et al.* (2000) explained about the amplification of *meq* gene for differentiation of oncogenic and attenuated strains of MDV-1. Krol *et al.* (2007) used both *meq, 132bp repeat* amplifications as duplex PCR for identification of oncogenic MDV-1 strains from vaccine strains. Hence this property is exploited in this study to determine the presence of oncogenic strains of MDV serotype 1 in Tamil Nadu poultry populations.

Present address: [1]SRF, [2]Associate Professor (email: drparthiban66@yahoo.co.in), [3]P G Student, [4]Professor and Head, Department of Animal Biotechnology**,** Madras Veterinary College, Chennai 600 007.

[5] Professor ADL VCCRI, Namakkal.

MATERIALS AND METHODS

Collection of samples: Clinical samples (60; feather follicles, liver and spleen) from chickens suspected of Marek's disease were collected from the Avian Disease Laboratory, Namakkal, Tamil Nadu. MDV serotype 1 available in our department (confirmed using commercial MDV Serotype 1 specific Monoclonal antibody) was used as positive control. HVT vaccine (serotype 3) was used as negative control.

DNA extraction: The feather tips were cut and immersed overnight at 55°C in 1 ml of the proteinase K buffer (0.5% sodium dodecyl sulfate [SDS], 0.1 M NaCl, 10 mM Tris pH 8.0, 1 mM ethylene diaminetetraacetic [EDTA]) containing proteinase K at a final concentration of 400 µg/ml. Total cellular DNA was extracted with 1 ml of phenol-chloroform-isoamyl alcohol (25: 24: 1), and centrifuged at 13 000 rpm for 15 min. To the upper aqueous phase equal volume of isopropanol was added and centrifuged at 13 000 rpm for 15 min and finally DNA was precipitated with 70% ethanol. The pellet was air dried and 100µl of nuclease free water was added to it.

Oligonucleotide primers used in duplex PCR: Two pairs of primers specific for the *Meq* gene and *132 bp repeats*

sequence (Krol *et al.* 2007) were synthesized (IDT make) and used.

Meq	FP	5′GCA CTC TAG AGT GTA AAG AGA TGT CTC AG 3′
Meq	RP	5′TAA CTC GAG GAG AAG AAA CAT GGG GCA TAG 3′
132bp repeats	FP	5′TAC TTC CTA TAT ATA GAT TGA GAC GT 3′
132bp repeats	RP	5′GAG ATC CTC GTA AGG TGT AAT ATA 3′

Duplex PCR: The reaction mixtures were prepared in a total of 25.0 µl volume consisted of Red dye master mix – 12.5 µl, *meq* FP (10 picomoles/µl) –1.0 µl, *meq* RP (10 picomoles/µl) –1.0 µl, *132bp rep* FP (10 picomoles/µl) –1.0 µl, *132bp rep* RP (10 picomoles/µl) –1.0 µl, DNA template (1µg/ml) –2.0 µl and nuclease free water – 6.5 µl. The amplification of both *Meq* gene and *132bp repeat* sequence was carried with denaturation at 94°C for 2 min followed by 35 cycles each consisting of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, after the final cycle, the elongation phase at 72°C was extended to 10 min. The PCR products were analyzed using 1.5% agarose gel. Following this the PCR products were purified and sequenced.

Nucleotide sequencing and BLAST analysis: The sequences that were obtained after sequencing in a genetic analyzer available in this department were subjected to BLAST analysis. The forward and reverse primers were used separately for sequencing and both the sequences were aligned using Gene tool software to obtain full length sequence of meq gene of MDV.

RESULTS AND DISCUSSION

Out of the 60 samples collected from Avian Disease Laboratory, Namakkal, Tamil Nadu, 8 samples were found positive by duplex PCR. The expected amplicon size of 1.06 kb for the *Meq* gene and 434 bp for *132 bp repeat sequence* were observed in 1.5% agarose gel. Positive control showed 2 bands of expected size and no bands were found in DNA samples of negative control (Fig. 1). The purified PCR products of *meq* gene were sequenced. The sequencing data matched 100% homology with other *meq* sequences available in the database on BLAST analysis (Accession numbers: EF523390.1, FJ436096.1, FJ436097.1).

The traditional diagnosis of MD is based on the clinical signs and pathological alteration. However, more specific methods for surveillance of MDV would be desirable to differentiate oncogenic strains from that of non-oncogenic strains. The different serotypes can be differentiated by the agar gel precipitation test (Lee *et al.* 1983), however the sensitivity of the test is inferior to that of enzyme linked immunosorbent assay (ELISA) and DNA hybridization (Davidson *et al.* 1986). Hence a duplex PCR technique was used for identifying virulent MDV serotype 1 strains.

Silva (1992) differentiated pathogenic and non-pathogenic serotype-1 MDV by amplification of the 132 bp tandem repeats within the MDV genome. The PCR was specific for serotype-1 MDV amplifying fragments corresponding to 1 to 3 copies of tandem repeats present in MD viruses. Chang *et al*. (2002) developed nested PCR assay to detect *meq* gene in chicken infected with serotype 1. Krol *et al.* (2007) used duplex PCR for detection of *meq* gene and *132bp sequence* of Marek's disease of pathogenic and vaccine strains of MDV serotype 1. However in India, HVT strain (serotype 3) is commonly used for vaccination. Hence in this study, duplex PCR was used for differentiation of field strains of MDV and HVT vaccine strain.

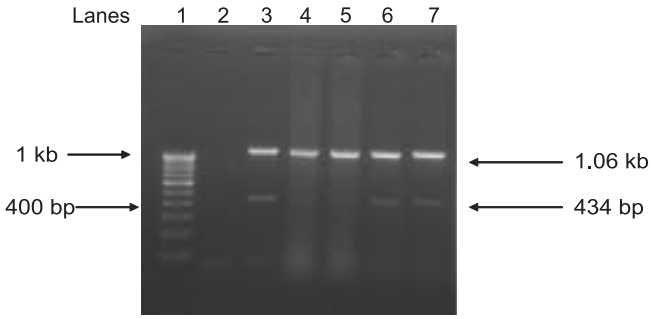

Fig. 1. Duplex PCR for identification of serotype 1 MDV. Lane 1, 100 bp DNA ladder; lane 2, negative control (serotype 3 HVT DNA); lanes 3–6, field samples (amplification of *Meq* gene and *132 bp repeat sequence*); lane 7, positive control (serotype 1 MDV DNA).

Duplex PCR carried out in the present study employs 2 pairs of primers targeting *meq* gene and *132 bp repeats sequence* to determine the prevalence of oncogenic MDV in the field. The presence of bands at 1.06 kb (*Meq*) and 434 bp (*132bp repeat sequence*) indicated the prevalence of MDV serotype 1 whereas no band was observed when duplex PCR was carried out with DNA extracted from HVT strain serotype 3. These results strongly support the use of PCR as an effective tool to determine the prevalence of serotype 1 MDV in Tamil Nadu poultry populations.

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