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Molecular detection of Marek's disease virus-1 (MDV-1) in the commercial broiler chicken of Tamil Nadu, India

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Abstract

The prevalence of Marek's disease virus-1 (MDV-1) was analysed from 60 flocks of commercial broiler chicken of Tamil Nadu, India which were not vaccinated against the MDV. The feather follicle epitheliums (FFE) were collected randomly from 1-15 days and 16-40 days age group and all the samples were subjected to Polymerase Chain Reaction (PCR) by amplifying the MDV-1 specific Meq gene. Among the overall prevalence of 20 per cent, none of the samples were showed amplification of MDV-1 Meq gene in 1-15 days age group, whereas 33.36 per cent of positivity was noticed in 16-40 days age group. At present, no MDV vaccination is practised in commercial broilers in India. Albeit, no gross lesions were reported within 40 days of its marketing age, but the confirmation of prevalence in FFE may be source of MDV to other susceptible chickens. Hence, vaccination to broilers can only reduce the spread of MDV-1.

Keywords: MDV-1, Meq, feather follicle, prevalence, PCR

Introduction

Marek's disease (MD) is a lymphoproliferative disease caused by Marek's Disease Virus-1 (MDV-1), a cell associated virus belonging to *Mardi virus* genus and Herpesviridae family [1,2]. MDV is classified into three serotypes, among three, Serotype 1 is a pathogenic strain possess oncogenic property (Rispen CVI988 vaccine strain), serotype 2 is a apathogenic strain isolated from chickens (SB1 and 301/B vaccine strain) and serotype 3 is the herpesvirus isolated from turkeys (HVT FC 126 vaccine strain) [3,4]. MD serotype 1 causes multiple T-cell lymphoma formation in the viscera, muscle, and skin as well as lesions in peripheral nervous tissues [5]. It occurs in chickens of 3-4 weeks of age or older and is the most commonly found in chickens between 12 and 30 weeks of age [6].

The transmission of the disease occurs through lateral transmission; direct or indirect contact between birds, inhalation of infected dust containing contaminated dander, and following a complex life cycle, the virus is shed from the feather follicle of infected birds [7]. Polymerase chain reaction (PCR) was considered to be rapid, highly sensitive and more specific for the detection of MDV-1 and enables differentiation of oncogenic and non-oncogenic strains of serotype 1 MDV and MDV vaccine strains of serotypes 2 and 3 [8, 9]. Detection of MDV in clinically affected and apparently healthy birds is helpful to know the presence of virus in poultry flock and institute appropriate prevention and control measures against it.

The disease causes significant economic loss to the commercial chicken farms and the estimated annual loss was up to US \$2 billion worldwide due to the mortality of 10-15% or beyond and can occur over a few weeks or many months [10, 11]. The unpredictability of MD outbreaks and the possibility of vaccination failure as a consequence of the evolution of more virulent strains of MDV, MD remains a major concern for the poultry industry [12]. As per the recent survey, the growth rate of 8.51% in egg and 7.52% in broiler production were noticed in the Indian poultry industry [13] where as the agriculture crops growth rate was 2.9% [14]. Estimates from the All India Poultry Breeders Association indicates that poultry contributes for USD 17.31 billion of total India's gross value and satisfies the hungers of 50 million people through direct and indirect employment.

Within the poultry sector, broiler and layer segment constitutes about 65.30% and 34.70% with the monthly turnover of 400 million chicks and 8,400 million eggs, respectively [15]. As per 20th census of livestock, Tamil Nadu is holding largest poultry population (120.8 million) in India.

The aim of this study is to detect the presence of MDV-1 specific gene in commercial broilers using PCR in Tamil Nadu, India to formulate the protocol for prevention and control of the disease, since the highest population of poultry in the state and frequent movement of the birds between states may act as source of virus to other susceptible chicken

Materials and Methods

Fifty commercial farms consist of 60 flocks from Namakkal, Perambalur, Ariyalur, Erode, and Tirupur district were selected with 12 flocks in each district. The flocks were divided into two age groups consist of 1-15 days and 16-40 days.

Sample collection

Five birds were randomly selected from each flock and the FFE samples were collected and pooled into a sample collection vial by noting the flock and collection date details. Totally 60 samples consist of 27 samples and 33 samples from 1-15 days and 16-40 days age group respectively. The samples were kept at 4 °C refrigerator temperature until further processing.

Sample preparation and DNA extraction

The pooled FFE samples were cut down around 3mm length and placed in a 1.5ml effendorf tube and processed the samples for DNA extraction using Blood and tissue DNA extraction kit (Qiagen, Germany) as per the manufacturer's

guidelines. The extracted DNA samples were kept at -20 °C until for further processing.

Amplification of serotype 1 MDV-specific gene using PCR

The PCR was carried out at Disease Investigation Laboratory, Department of Veterinary Preventive Medicine, Veterinary College and Research Institute, Namakkal. A pair of primers specific to the MDV genome region encoding the *meq* gene were used with the sequences of 5'- GGC-ACG- GTA-CAG – GTG- TAA- AGA- G -3' for the forward *Meq* primer and 5'- GCA-TAG-ACG –ATG- TGC- TGC- TGA- G -3' for the reverse *Meq* primer with amplification size of 1081bp [16]. The PCR reaction was carried out as per earlier described protocol for a final volume of 50 µl with slight modifications including 5 µl of template DNA, 25 µl of 1x Master Mix (Amplicon), 20 pmol of each forward and reverse primers and 20 µl of nuclease free water and the amplification was performed using Mastercycler (Effendorf, Germany) with initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 40 s for each cycle, and a final extension at 72 °C for 10 min. [16]. The PCR products were analyzed by separating the products by gel electrophoresis in 1.65% agarose gel containing Ethidium bromide. Finally, the gel was analyzed using Medox gel documentation Imager (Medox, India). The chi-square test was used to detect the significance between different age groups.

Results and Discussion

The MDV-1 positive samples were shown the amplification at 1081bp (Fig.1). Among 60 FFE samples tested by conventional PCR, the positive for MDV-1 was 12 samples.

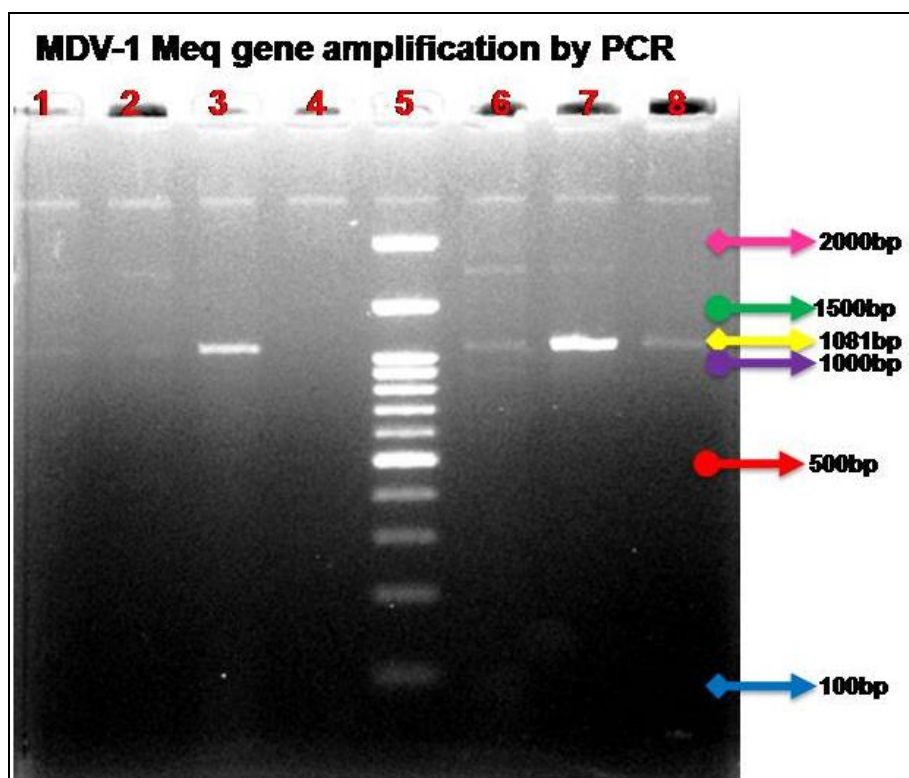


Fig 1: Lane 4: Negative Control and Lane 5: 100bp Ladder Lane 1,2,3,6,7,8- FFE samples

Out of 27 samples tested in the 1-15 days age group, none of the samples had shown positivity where as 12 samples were found positive among 33 samples tested in the age group of

16-40 days and significant difference was noticed in the prevalence between two age groups (Table.1).

Table 1: District wise and Age wise Prevalence of MDV-1 in commercial Broilers in Tamil Nadu, India

Place	1-15 days		Positives (in %)	16-40 days		Overall Positives (in %)	Overall prevalence (in %)
	No. of Samples	No. of Positives		No. of Samples	No. of Positives		
Ariyalur	4	0	0	3	0	0.00	0.00
Erode	3	0	0	5	1	20.00	12.5
Namakkal	5	0	0	7	4	57.14	33.33
Perambalur	5	0	0	6	1	16.67	9.09
Tirupur	10	0	0	12	6	50.00	27.27
Total	27	0	0	33	12	36.36	20.00

* Significant difference between the age group ($P < 0.5$)

As the MD could occur in any age beginning at 3-4 weeks which is agreed with the present study [6]. The overall prevalence was found 20 per cent in the commercial broilers of Tamil Nadu, India which is coincided with the earlier findings in the vaccinated layer chicken farms of Tamil Nadu [17]. However, other studies reported higher rates of prevalence of MD in poultry farms viz., MDV-1 was detected by PCR in spleen tissue from all the flocks at rates varying between 10% and 70% and in feather tip extracts at rates varying between 60% and 100% [18]. In the present study, the MDV-1 prevalence was noticed more in Namakkal and Tirupur district of Tamil Nadu where the commercial poultry birds were densely populated. The higher prevalence in those places might be due to transmission of MDV-1 between commercial layer and broiler birds and vice versa. As per the earlier report, MDV genetic material was most often found in broiler chickens (49 flocks; 69.01%), and then in descending order in layer breeders (7 flocks; 9.85%), in commercial layers (5 flocks; 7.04%) and broilers turkeys (1 flock; 1.40%) and broiler geese (1 flock; 1.40%) [19]. The present study found 36.36 percent of MDV-1 *meq* gene positivity in the age group of 15-40 days which is agreed with findings of recent report, in Poland during 2017 viz., 2- 6 weeks aged bird was shown more positive in the 28 flocks of broiler chickens [19]. The frequent transportation of broiler birds between different districts and different states might act as source of the virus to the MD non affected areas. Hence, an appropriate prevention and control measures may be warranted to avoid the disease spread.

Conclusion

The present study has confirmed the presence of MDV-1 prevalence in the commercial broilers of Tamil Nadu, India. As the commercial broilers are marketed within 40 days of age in which the chances of symptomatic MD might be very less. Hence, the broilers are not vaccinated against MDV still today. Further, it is revealed that the presence MDV-1 oncogenic gene (*meq*) without vaccination in the commercial broilers is the major threat in the transmission of the disease between different types of chicken reared in the country. Albeit no pathological lesions were not noticed due to its early age marketing, but the feathers would act as main source of virus to other susceptible chickens. Hence, the vaccination to the broilers is the only solution to reduce the spread of MDV-1.

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