Journal of Animal Research: v.11 n.4, p. 649-652. August 2021

DOI: 10.30954/2277-940X.04.2021.11

Molecular Identification of Marek's Disease Virus in Vaccinated Commercial Layers of Tamil Nadu in India

M. Saravanajayam[1*], K.M. Palanivel[1], G. Selvaraju[2], A. Balasubramaniam[3] and P. Srinivasan[4]

[1]Department of Veterinary Preventive Medicine, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, INDIA

[2]Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Namakkal, TANUVAS, Tamil Nadu, INDIA

[3]Avian Disease Laboratory, Thalaivasal, TANUVAS, Tamil Nadu, INDIA

[4]Department of Veterinary Pathology, Veterinary College and Research Institute, Salem, TANUVAS, Tamil Nadu, INDIA

**Corresponding author: M Saravanajayam; E-mail: saravet78@gmail.com*

Received: 14 Jul, 2021 **Revised:** 28 July, 2021 **Accepted:** 30 July, 2021

ABSTRACT

We report the presence of Marek's disease (MD) virus *meq* among commercial layer chickens with or without the history of MD outbreak. Feather follicle epithelial (FFE) samples (285) were collected from 50 vaccinated commercial layer flocks of Tamil Nadu in India regardless of MD outbreak and were subjected to polymerase chain reaction (PCR) to amplify MDV specific *meq* gene. The prevalence of MD was found to be 50 per cent. Among 285 feather follicle samples tested from different age groups, the highest prevalence was noticed at 17 to 40 week age groups (38.03 per cent) followed by 9 to 16 week age groups (33.33 per cent), 41 to 60 week (32.26 per cent) age groups, 61 week and above (32.14 per cent) age groups and 0 to 8 week (23.68 per cent) age groups with overall prevalence of 32.63 per cent. The highest prevalence was noticed in the peak production period which might be attributed to activation of latent virus. It is suggested that the MD prevalence in twice vaccinated layer flocks warrants for stringent control measures in order to check the re-emergence of MD among layer chicken.

HIGHLIGHTS

- Marek's disease prevalence was detected in the vaccinated commercial layer chicken.
- Feather follicle epithelium samples were used to amplify MDV *meq* gene by PCR.
- The highest prevalence was recorded in the peak egg production group.

Keywords: Marek's disease, *meq* gene, prevalence, FFE, PCR

Marek's disease (MD) is one of the virus induced neoplastic disease of chicken caused by Mareks disease virus serotype 1 (MDV1), belongs to Mardi virus genus. The direct economic losses was due to hen mortality and morbidity (e.g., egg production loss), and indirect losses due to wide use of vaccines and expenses spent on control measures (Rozins *et al.,* 2019). The MDV consists of three serotypes and among that serotype 1, 2 and 3 are an oncogenic to chicken, non-pathogenic to chicken and herpesvirus of turkey respectively. MDV1 possess a specific *meq* oncogene which is expressed only in serotype 1. Hence, the molecular identification of MDV1 *meq* gene directly shows the prevalence of MD. Feather follicle epithelium (FFE) is the only anatomical site for cell free MDV from where it spreads horizontally in poultry houses with dust and dander and also productive replication occurs in FFE. The FFE could be used as supporting organ in the molecular diagnosis of MDV in commercial chicken

How to cite this article: Saravanajayam, M., Palanivel, K.M., Selvaraju, G., Balasubramaniam, A. and Srinivasan, P. (2021). Molecular Identification of Marek's Disease Virus in Vaccinated Commercial Layers of Tamil Nadu in India. *J. Anim. Res.,* **11**(04): 649-652.

Source of Support: None; **Conflict of Interest:** None

flocks (López- Osório *et al.,* 2019). The MD was confirmed using molecular techniques (PCR) recently in desi chicken and commercial broilers in Tamil Nadu (Balasubramaniam *et al.,* 2017; Saravanajayam *et al.,* 2021). Further, there is predominantly visceral form of MD which occurs after onset of lay in the layer chicken for the past two decades, hence, the study was taken to know the real prevalence of MD in commercial layer chicken which will be useful to make prevention and control strategies.

MATERIALS AND METHODS

Fifty commercial layer chicken farms comprising of 285 flocks were selected for random sampling of the feather follicle epithelium (FFE) in Tamil Nadu to assess the prevalence of the MD. The sampling was done with pre-prepared epidemiological questionnaire seeking age, production status, vaccination schedules followed and previous MD outbreaks, if any. All the layer flocks were vaccinated with MDV cell associated vaccine at zero day in hatchery and day-old at farm premises. The FFE samples with the length of ~3 mm were cut into small pieces using sterile scissors and labelled in a 1.5 ml micro centrifuge tube for DNA extraction. The DNAesy Blood and Tissue kit (Qiagen, Germany) was used to extract DNA from FFE samples. The DNA quantity and quality were estimated by the OD ratio at 260:280 nm using Nanodrop (Thermo scientific, USA) method. The samples having the acceptable purity ratio between 1.6 - 1.8 were quantified and used for PCR.

The primers used to amplify *meq* gene with the size of 1081 bp were 5'- GGC-ACG-GTA-CAG-GTG-TAA-AGA-G – 3'(Forward) and 5'- GCA-TAG-ACG-ATG-TGC-TGC-TGA-G– 3' (Reverse). The PCR reaction mixture was prepared using 12.5 µl of Taq polymerase master mix (2X), 20 *pmol* of both forward and reverse primers, 2 µl of template DNA and 8.5 µl of nuclease free water to the final volume of 25 µl in the 200 µl PCR. The PCR tubes containing the reaction mixture were kept in the thermal cycler (PCR Mastercycler ep gradient S, Effendorf, Germany) and the amplification of MDV-1 *meq* gene was carried out as per the method described by Tian *et al.* (2011). Briefly, initial denaturation at 94ºC for 4 min followed by 35 cycles of denaturation (94ºC for 1 min), annealing (56ºC for 1 min) and extension (72ºC for 90 sec) and final extension with 72ºC for 10 min. The visualization of the *meq* gene was performed in the 1.65% agarose gel with 1X TAE buffer and ethidium bromide. The PCR products were loaded with quantity of 5 µl in each well along with one well consist of same quantity of 1500 bp DNA molecular weight marker (Bio-Rad, USA) and were electrophoresed at 80V for 40 min. The amplified products were placed in the Gel documentation system (Medox, India) for visualization and documentation.

The Chi-square test was used to analyze the significance in the molecular detection of Marek's disease virus in commercial layer chickens.

RESULTS AND DISCUSSION

The MDV *meq* PCR amplification showing 1081 bp positive was depicted in the Fig. 1.

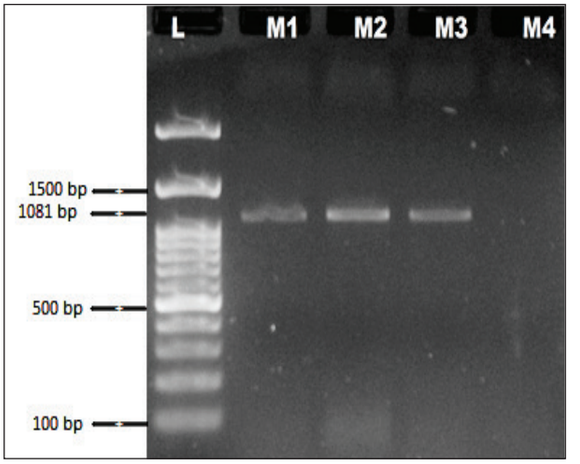

Fig. 1: PCR amplification of MDV1 *meq* gene (1081 bp) from FFE sample of commercial layer chicken. **Lane**-L – 1500 bp DNA molecular weight marker; **Lane**- M1 to M4 – Field Samples; M1 to M3 – Positive for *meq* gene M4-Negative for *meq* gene

Among the MDV vaccinated commercial layer farms, the prevalence of MDV was observed 50 per cent from farms with and without history of MD outbreak and with current MD outbreak. The *meq* gene prevalence has been considered as the real prevalence of Marek's disease in the farms, since *meq* gene could express in all stages of MD pathogenesis. Among MD prevalence in the commercial layer, Namakkal area of Tamil Nadu was showed highest prevalence of 55.17 per cent followed by Attur, Erode and Annur area (Table 1 and 2). Similarly, Tian *et al.* (2011)

and Zhang *et al.* (2011) reported the prevalence of MD mortality of 15 to 60 percent in vaccinated breeder and layer flocks whereas the prevalence of MD was 26 per cent in unvaccinated flocks (Brown *et al.,* 2013). Witter (1996 and 1997) reported the higher incidence of MD in bivalent (HVT+SB1) vaccinated flocks which is congruous to the present observation.

Table 1: Molecular prevalence of MD in vaccinated commercial layer farms

Farm Status	No. of farms	Overall prevalence	
		No. of positives	Percentage
With History of MD outbreak	32	14	43.75
With current MD outbreak	8	8	100.00
No History of outbreak	10	3	30.00
Total	**50**	**25**	**50.00**

*Significant difference in the MD prevalence between farms of different disease status (p<.05).

Table 2: Area-wise molecular prevalence of MDV-1 in vaccinated commercial layers in Tamil Nadu

Area	No. of the farms	MDV *meq* +ve	MDV *meq* + %
Namakkal	29	16	55.17
Erode	10	4	40.00
Attur	6	3	50.00
Annur	5	2	40.00
Total	**50**	**25**	**50.00**

*No significant difference in the MD prevalence between different area of Tamil Nadu (p>.05).

Among different age groups, the highest prevalence was noticed at 17 to 40 week age groups (38.03 per cent) followed by 9 to 16 week age groups (33.33 per cent), 41 to 60 week (32.26 per cent) age groups, 61 week and above (32.14 per cent) age groups and 0 to 8 week (23.68 per cent) age groups with overall prevalence of 32.63 per cent (Table 3).

The highest prevalence in layers at age group 17 to 40 weeks was also reported by Reddy *et al.* (1980); Panda *et al.* (1983); Arulmozhi *et al.* (2011) and as they found the highest prevalence at 26 week, 21 to 40 week and 28 to 38 week of age groups respectively. The highest prevalence is due to the stress during peak egg production in the age group of 17 to 40 weeks which is in the agreement with the findings of Zuang *et al.* (2015). The lowest prevalence at 0 to 8 weeks are in contrast with report of Garg *et al.* (1979); Panda *et al.* (1983) and Haribabu (1986) who were reported the lowest prevalence in 9 to 20 week, 3 to 5 and 5 month of age groups respectively. Dense population, vaccination failure, virus evolution to overcome vaccine response and poor management might be contributing factors for the prevalence of MD in vaccinated commercial layers.

Table 3: Age-wise prevalence of Mareks' disease in vaccinated commercial layers

Age of the birds (weeks)	No. of FFE samples	No. of samples positive	Percentage of positives
0 - 8	38	9	23.68
9 - 16	27	9	33.33
17 - 40	71	27	38.03
41 - 60	93	30	32.26
61 and above	56	18	32.14
Total	**285**	**93**	**32.63**

*No significant difference in the MD prevalence between different age group (p>.05).

However, the commercial layer chicken are vaccinated with bivalent vaccine at zero day age in hatchery and day-old at farm premises, still the MD occurrence is in the higher side, hence the changing of vaccination strategy via *in ovo* technique and make use of the serotype 1 vaccine may give better solution for the prevailing situation in the layer industry.

ACKNOWLEDGEMENTS

The authors are thankful to the Tamil Nadu Veterinary and Animal Sciences University, Chennai to carry out this research work.

REFERENCES

Arulmozhi, A., Saravanan, S., Mohan, B. and Balasubramaniam, G.A. 2011. Marek's disease in vaccinated poultry flocks in and around Namakkal region of Tamil Nadu. *Indian J. Vet. Pathol.,* **35**(1): 45-47.

Balasubramaniam, A., Saravanajayam, M. and Arulmozhi, A. 2017. Recurrent Incidence of Marek's Disease in Native Breed Chickens. *J. Anim. Res.,* **7**(4): 789-791.

Brown, S.W.W., Islam, A., Groves, P.J., Rubite, A., Sharpe, S.M. and Burgess, S.K. 2013. Development, application, and results of routine monitoring of Marek's disease virus in broiler house dust using Real-Time quantitative PCR. *Avian Dis.,* **57**(2s1): 544-554.

Garg, S.K., Singh, I.P., Rao, V.D.P. and Chandra, R. 1979. Incidence of Marek's disease precipitins in commercial poultry farms. *Indian Poult. Rev*., **10**: 11-13.

Haribabu, Y. 1986. Seroprevalence of Marek's disease on gel diffusion test. *Indian J. Poult. Sci.,* **21**: 240-242.

López-Osorio, S., Villar, D., Piedrahita, D., Ramírez-Nieto, G., Nair, V., Baigent, S.J. and Chaparro, G. 2019. Molecular detection of Marek's disease virus in feather and blood samples from young laying hens in Colombia. *Acta Virol.,* **63**(4): 380-391.

Panda, B.K., Arya, S.C., Pradhan, H.K. and Johari, D.C. 1983. Marek's disease incidence in various strains of white leghorn chickens. *Indian J. Poult. Sci.,* **18**: 100-103.

Reddy, S.D., Pargaonkar, V.N. and Moorthy, K.G. 1980. Serological survey of Marek's disease by agar gel precipitation test. *Indian Vet. J.,* **57**: 185-189.

Rozins, C., Day, T. and Greenhalgh, S. 2019. Managing Marek's disease in the egg industry. *Epidemics,* **27**: 52-58.

Saravanajayam, M., Palanivel, K.M., Selvaraju, G., Srinivasan, P. nd Balasubramaniam, A. 2021. Molecular detection of Marek's disease virus- 1 (MDV-1) in the commercial broiler chicken of Tamil Nadu, India. *J. Entomol. Zool. Stud*., **9**(1): 1571-1574.

Tian, M., Zhao, Y., Lin, Y., Zou, N., Liu, C., Liu, P. and Huang. Y. 2011. Comparative analysis of oncogenic genes revealed unique evolutionary features of field Marek's disease virus prevalent in recent years in China. *Virol. J.,* **8**: 1-11.

Witter, R.L. 1997. Increased virulence of Marek's disease virus field isolates. *Avian Dis.,***41**: 149–163.

Zhang, Y.P., Chang-jun, L., Feng, Z., Weisong, S. and Jingmei, L. 2011. Sequence analysis of the *meq* gene in the predominant Marek's disease virus strains isolated in China during 2006–2008. *Virus Genes,* **43**(3): 353.

Zhuang, X., Zou, H., Shi, H., Shao, H., Ye, J., Miao, J., Wu, G. and Qin, A. 2015. Outbreak of Marek's disease in a vaccinated broiler breeding flock during its peak egg-laying period in China. *BMC Vet. Res.,* **11**:157.