Comparision of Polymerase Chain Reaction and Agar Gel Immunodiffusion test in Detection of Marek's Disease Virus

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Abstract

A study was undertaken to identify Marek's disease virus (MDV) antigen by PCR and AGID and to test the significance of PCR and AGID by McNemar's test in detection of MDV antigen in outbreak in layer flocks. A total of twelve different MD outbreak flocks with varying flock size were selected in this study. Feather follicles were collected from 10 apparently healthy birds, 10 clinically affected birds and 10 dead birds separately in each outbreak. All the samples were subjected to PCR and AGID. In PCR, 42 (35.00%), 68 (56.67%) and 106 (88.33%) samples were positive to MDV in apparently healthy birds, clinically affected birds and dead birds respectively and in AGID 28 (23.33%), 56 (46.67%) and 98 (81.67%) samples were positive to MDV in apparently healthy birds, clinically affected birds and dead birds respectively. In testing the significance of PCR and AGID in detecting MDV, significant difference existed between the two tests in feather tips of apparently healthy birds ($P < 0.05$), whereas there was no significant difference between PCR and AGID in detection of MDV in feather tips of clinically affected and dead birds ($P > 0.05$). Hence, PCR can be used to screen MDV in apparently healthy birds and AGID can be used to screen MDV in clinically affected and dead birds keeping feasibility and economic consideration.

Keywords: Marek's disease, Herpesvirus of turkey, Polymerase chain reaction, Agar gel immunodiffusion test

Introduction

Marek's disease, a disease of chicken characterized by lymphoproliferation and neoplasia of lymphoid tissue, continues to be a disease of major economic importance in commercial flocks throughout the world (Witter, 1971). It is caused by herpesvirus belonging to serotype 1 Marek's disease virus (MDV) (Witter, 1998). Morbidity and mortality due to MD was receded by introduction of HVT vaccine in 1970, but vaccine breaks began to be reported and increased virulence of challenge viruses occurred within ten years (Pastoret, 2004).

Chickens of different ages are kept and raised in the same house at the same time and continuous production makes proper sanitation impossible and creates high level of infectivity in the house. In most of the commercial flocks, vaccine is administered at hatchery and within a few hours of vaccination the chickens are placed in brooder house, where exposure to environmental MDV is likely. 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. Polymerase chain reaction is highly sensitive test (Silva, 1992 and Davidson et al., 1995) in detecting MDV, but it requires costly equipments and chemicals. Marek's disease viral antigen can be detected in the feather follicle by the agar gel precipitation test (OIE, 2004; Kamaldeep et al., 2007; Palanivel et al., 2007) which is a simple and sensitive technique (Marquardt, 1972).

Keeping this in view, the study is envisaged with the following objectives,

i) Molecular and serological detection of Marek's disease virus (MDV) from apparently healthy, clinically affected and dead birds.

ii) Comparison of polymerase chain reaction and agar gel immunodiffusion test (AGID) in detection Marek's disease virus.

Materials and Methods

The MDV antigen being maintained in the department was used as reference antigen. The MDV hyperimmune serum was raised in ten, four weeks old

Table-1. Positivity to MDV by PCR and AGID

Test	Apparently healthy birds		Clinically affected birds		Samples ofDead birds	
	No. Positive	Per cent	No. Positive	Per cent	No. Positive	Per cent
PCR	42	35.00	68	56.67	106	88.33
AGID	28	23.33	56	46.67	98	81.67

cockerels and used as reference antiserum. Three sets of primer pairs were selected as suggested by Becker et al. (1992) and were custom synthesized (GeNei, Bangalore). The sequence of the primers were as follows:

Common primer for MDV-1 and MDV-3

AGA1(F) ATACCACGCCAACGAAAAGAATGT
AGA1.8 (R) CTATAGTACATATTGCATACCCAT
Specific primer for MDV-1
BAMH1(F) TACTTCCTATATAGATTGAGACGT
BAMH2(R) GAGATCCTCGTAAGGTGTAATATA
Specific primer for MDV-3
HVT- 1 (F) ATGGAAGTAGATGTTGAGTCTTCG
HVT-2 (R) CGATATACACGCATTGCCATACAC

A total of twelve different MD outbreak reported flocks with varying flock size were selected in this study. Feather follicles were collected from 10 apparently healthy birds, 10 clinically affected birds and 10 dead birds separately in each outbreak.

A total of 120 samples were subjected to AGID, as per the method described by OIE (2004). After 24 – 48 hours of incubation, the AGID slides were stained with Amidoblack stain as per the standard procedure. The same 120 samples were subjected to PCR as per the method described by Handberg et al. (2001) with three different set of primers. Testing the significance of AGID and PCR in detection of MDV antigen is carried out by McNemar's test (Armitage and Berry, 1987).

Results and Discussion

Positivity to MDV by PCR and AGID is shown in the table-1.

Detection of MDV by PCR

In this study, 42 (35.00%), 68 (56.67%) and 106 (88.33%) samples were positive to MDV in apparently healthy birds, clinically affected birds and dead birds respectively. Both common primer (AGA1/AGA1.8) and serotype 1 specific primer (BamH1/BamH2) amplified A gene of MDV, AGA1/AGA1.8 produced band size of 686 bp and serotype 1 specific primer (BamH1/BamH2) amplified 132 bp tandem repeats, producing expected band size of 434 bp. None of the field samples were amplified by serotype 3 specific primer (HVT-1/HVT-2). The BamH1/BamH2 amplified only MDV-1, but not MDV-2 or MDV-3. The HVT-1/HVT-2 amplified only MDV-3, but not MDV-1 or MDV-2. Becker et al. (1992) also reported similar type of findings. Baigent et al. (2006) used PCR to examine various aspects of vaccination in experimental chicks and commercial chicks with a view to determine how vaccine level in feather correlate with protection against challenge and for identifying optimal timing, vaccine delivery route and optimal vaccination regimes for different breeds of chicks.

Detection of MDV by AGID

In this study, 28 (23.33%), 56 (46.67%) and 98 (81.67%) samples were positive to MDV in apparently healthy birds, clinically affected birds and dead birds respectively. Vathsala and Mohan (2006) also observed that 56.41 per cent positivity in MD outbreak reported flock by AGID. Soluble antigen A was detected only from feather tips infected with MDV-1, but not HVT because of poor replication of HVT in feather tips and poor expression of soluble A antigen. Hence, AGID can be used to distinguish MDV-1 from HVT. This is in agreement with the findings of Bulow and Biggs (1975); Rangga-Tabbu and Cho (1982).

Comparison of PCR and AGID in detecting MDV

Testing the significance of AGID and PCR in detection of MDV antigen by McNemar's test is shown in the Table 2. In apparently healthy birds, calculated value 2.33* is grater than the tabulated value at 0.05 level ($P < 0.05$). In clinically affected birds, calculated value 1.73NS is less than the tabulated value at 0.05 level ($P>0.05$). In dead birds, calculated value 1.16NS is less than the tabulated value at 0.05 level ($P>0.05$). This result indicates that significant difference exists between PCR and AGID in detection of MDV in apparently healthy birds of MD outbreak flocks, moreover PCR (35.00%) had higher positivity than

Table – 2. Testing the significance of AGID and PCR in detection of MDV antigen by McNemar's test

Typeof	Diagnostic tests		Number of pairs in		
pairs	PCR	AGID	Apparently healthy birds	Clinically affected birds	Dead birds
1	Positive	Positive	13	25	45
2	Positive	Negative	8	9	8
3	Negative	Positive	1	3	4
4	Negative	Negative	38	23	3

AGID (23.33%). In clinically affected and dead birds of MD outbreak flocks, there is no significance difference between PCR and AGID in the detection of MDV.

Polymerase chain reaction can be used as potential tool for routine monitoring of flocks for early exposure to MDV as reported by Young and Gravel (1996). Moreover, PCR could be used to determine how vaccine level in feather correlates with protection against challenge and for identifying optimal timing, vaccine delivery route and optimal vaccination regimes for different breeds of chicks as said by Baigent et al. (2006). Agar gel immunodiffusion test as efficacious as PCR and it is quite simple to screen MDV in clinically affected and dead birds at filed level as suggested by OIE, 2004; Kamaldeep et al., 2007; Palanivel et al., 2007.

Acknowledgement

The authors express their sincere gratitude to the Dean, Veterinary College and Research Institute, Namakkal for providing needful facilities to carry out this study.

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