Recent Trends in Diagnosis and Control of Marek’s Disease (MD) in Poultry

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Abstract: Marek’s Disease (MD), caused by Marek’s Disease Virus (MDV) is a highly contagious oncogenic and neurotropic disease of chickens responsible for great economic losses to the poultry industry all around the world and characterized by development of CD4+T cell lymphomas as well as infiltration of nerves and visceral organs by lymphocytes. MD is one of the most common lymphoproliferative diseases of chickens which cause mononuclear cell infiltration in one or more of the following tissues: peripheral nerves, gonads, lymphoid organs, iris, muscle, skin and other visceral organs resulting into development of tumours in visceral organs, paralysis of legs, wings and neck, grey eye (iris) or irregular pupil, vision impairment, blindness, skin lesions and immunosuppression, all of which can be accompanied by non-specific signs such as anorexia, weight loss and poor performance. Today there are evolving highly pathogenic isolates of MDV around the world capable of overwhelming the protection from currently employed vaccines. Thus MD poses a big challenge to the welfare and wellbeing of the poultry with increased condemnation of carcass, loss of productivity and quality products, leading to huge economic losses. It is also an immunosuppressive disease and causes increased susceptibility to other infections. The present review discusses in brief about the Marek’s disease, its etiology, conventional and advance tools and techniques being used for its diagnosis, prevention and control strategies in poultry.

Key words: Marek’s disease, poultry, diagnosis, vaccine, prevention and control

INTRODUCTION

Marek’s Disease (MD) is caused by Marek’s Disease Virus (MDV) and it is a highly contagious oncogenic and neuropathic disease of chickens responsible for great economic losses to the poultry industry worldwide. Sporadic outbreaks of MD have been reported recently throughout the world even in vaccinated flocks (Powell and Lombardini, 1986; Kuna et al., 2001; Okwor and Eze, 2011; Lobago and Woldemeskel, 2004), including India (Rajkhowa, 2005; Bineesh et al., 2007; Jadhav et al., 2007; Kamaldeep et al., 2007; Raja et al., 2009; Arulmozhri et al., 2011; Gopal et al., 2012).

The disease is characterized by development of CD4+ T cell lymphomas as well as infiltration of nerves and visceral organs by lymphocytes.

Dr. Jozsef Marek first recognized the disease as a paralysis of roosters in the year 1907. MD almost devastated the poultry industry in the 1960s but the disease was brought under control after Marek’s disease Herpes Virus of Turkey (HVT) was identified and live vaccines were developed in 1970’s. Thereafter, variant MD viruses evolved with increased pathogenicity. Subsequently, many MD outbreaks have been reported worldwide and new vaccines developed to combat MD viruses with higher virulence. Earlier it is considered as paralytic disease but now-a-days, it is manifested as an acute disease with tumours in multiple visceral organs. Today there are evolving highly pathogenic isolates of MDV around the world capable of overwhelming the protection from currently employed vaccines. Thus, MD poses a big challenge to the welfare and wellbeing of the
poultry with increased condemnation of carcass, loss of productivity and quality products, leading to huge economic losses. It is also an immunosuppressive disease and causes increased susceptibility to other infections.

**ETIOLOGICAL AGENT (MDV)**

The causative agent of the diseases is Marek’s Disease Virus (MDV) and as per the recent classification by the International Committee on Taxonomy of Viruses (ICTV, 2011), it is placed in Order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Mardivirus* (Marek’s disease-like viruses). MDV is a cell associated herpes virus consisting of a linear, double stranded DNA of 160-180 kbp in size. MDV-Herpes virus group has been divided into three serotypes based on their biological properties viz. serotype 1, 2 and 3. Serotype 1 MDV is virulent and oncogenic whereas serotype 2 and 3 (HVT) are non-pathogenic vaccine strains. Serotype 1 MDV strain viruses are further classified into pathotypes (Witter *et al.*, 2005) based on induction of lymphoproliferative lesions and severity of disease in vaccinated chickens (Table 1).

**CONVENTIONAL MD DIAGNOSIS**

Primary diagnosis is based on age, clinical signs, history and gross and microscopic lesions. Diagnosis of MD is easier in general in chickens younger than 14 weeks of age, although non-bursal tumors could be caused by REV which must be excluded. However, REV non-bursal tumors are generally not recorded in commercial flocks below 14 weeks of age. Lymphoid leukosis is the most likely to be diagnosed in older birds with bursal tumors, but MD virus and REV both can cause bursal tumors. Occurrence of tumours in older birds in the absence of bursal tumors, MD is the most probable diagnosis. Grossly, the disease is characterized by paralysis of legs (Fig. 1), wings and neck, and tumour nodules in visceral organs (Fig. 2) depending upon the tissue or organ involved. Other observations include grey eye (iris) or irregular pupil, vision impairment, blindness, skin lesions and immunosuppression. Microscopically, mononuclear cell infiltration in one or more of the following tissues: peripheral nerves, gonads, lymphoid organs, iris, muscle, skin and other visceral organs (Fig. 3, 4, 5) is observed. Laboratory confirmation is done by virus isolation in susceptible (newly hatched) chicks, embryonated eggs and tissue cultures and subsequent identification (Kaur, 2005). MDV can be isolated in chicken embryos by yolk sac route (4-5 days embryo) and later examining their chorioullaotic membranes (CAM) on 18th day of incubation for the ‘pock lesions’ (whitish raised nodules). Infected embryos also show atrophy of muscles and curling. MD virus can also be isolated in Duck Embryo Fibroblast (DEF) and chicken kidney cell culture systems. After 5-14 days, plaque formation or cytopathic effects (CPE) are observed in cell culture. Type A intranuclear inclusion bodies can be noticed in infected cells. Serotype can be confirmed by using specific monoclonal antibodies. Viral antigen can be

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**Table 1: Classification of MDV serotypes and their representative strains**

<table>
<thead>
<tr>
<th>MDV serotypes</th>
<th>Pathotype or strains</th>
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<tbody>
<tr>
<td><strong>Serotype 1</strong></td>
<td></td>
</tr>
<tr>
<td>(Pathogenic or oncogenic strains as well as attenuated strain of these viruses)</td>
<td>Very virulent plus (vv+): 648A</td>
</tr>
<tr>
<td></td>
<td>Very virulent (vv): Md5, Md/11, Ala-8, RB-1B</td>
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<tr>
<td></td>
<td>Vireulant (v): HPRS-16, JM GA</td>
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<tr>
<td></td>
<td>Mild (m) vireulant: HPRS-B14, Con A</td>
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<tr>
<td></td>
<td>Weekly vireulant: CU-2, CVI-988</td>
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<tr>
<td><strong>Serotype 2</strong></td>
<td></td>
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<tr>
<td>(Naturally non-pathogenic, non-oncogenic or avirulent strains)</td>
<td>SB-1, HPRS-24, 301B/1, HN-1</td>
</tr>
<tr>
<td><strong>Serotype 3</strong></td>
<td></td>
</tr>
<tr>
<td>(Naturally avirulent strains, non-oncogenic)</td>
<td>HVT (FC126, P91)</td>
</tr>
<tr>
<td>(Herpes virus of Turkey)</td>
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**Fig. 1:** MD affected layer bird with unilateral leg paralysis displaying “sportsman-like” posture

**Fig. 2:** MD lesions in liver with numerous greyish-white coalescing tumour nodules
detected in feather tips, follicle epithelium and infected lymphoid tissue by Agar Gel Precipitation Test (AGPT), Fluorescent Antibody Technique (FAT), Immunoperoxidase Test (IFT) and Enzyme-linked Immunosorbent Assay (ELISA) (Schat and Nair, 2008). Demonstration of Marek's disease Associated Tumour Surface Antigen (MATSA) on the transformed cells can be of limited diagnostic value since activated T-cells also express this protein. Immunohistochemistry can be effectively used to demonstrate MDV proteins especially "Meq" oncoprotein which is consistently expressed in all MD tumours.

**RECENT ADVANCES IN MD DIAGNOSIS**

**Polymerase chain reaction (PCR)**

PCR: The full length genomic sequences of MDV 1 (GenBank accession numbers: strain Md5; AF243438, GA; AF147806, Md11; AY510475, CV1988; DQ530348), MDV 2 (GenBank accession numbers: strain SB-1; HQ840738, HPS24; ABO46735 (Izumiya et al., 2001) and MDV 3 (GenBank accession number: strain FC126; AF291866) are available now. This enables the PCR-based diagnostic methods for serotype specific detection of MDV. PCR tests enabling differentiation of oncogenic and non-oncogenic strains of MDV serotype and MDV vaccine strains of serotypes 2 and 3 (Beeker et al., 1992; Zhu et al., 1992; Hannberg et al., 2001). PCR tests to detect MDV 1 specific meq oncogene (Fig. 6) in feathers and tumours have been published (Lee et al., 2000; Chang et al., 2002).

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**Fig. 3:** Numerous foci of transformed lymphocytes in the parenchyma of liver. HE x100

**Fig. 4:** Pleomorphic lymphocytes infiltrating the parenchyma of kidney. HE x400

**Fig. 5:** MD lesions in sciatic nerve: marked mononuclear cells infiltration (Type A lesion). HE x400

**Fig. 6:** PCR for detection of 683 bp amplicon of MDV-1 genome
Nested PCR: Specific detection of meq oncogene of MDV 1 in infected spleen cells, feather tips and peripheral blood mononuclear cells by nested PCR have been developed (Lee et al., 2000; Murata et al., 2007).

Multiplex PCR: Simultaneous detection of MDV 1, avian leukosis virus and reticuloendotheliosis virus in tumour tissues of naturally infected chickens and turkeys has been developed using multiplex PCR (Gopal et al. 2012).

Quantitative real-time PCR (qPCR): qPCR to quantify MDV genome copies have been described for simultaneous detection and quantitation of viral load in clinical samples or infected tissues (Islam et al., 2004; Bagent et al., 2005; Abdul-Careem et al., 2006). Since MDV 1 is ubiquitous, its quantitation in suspected clinical samples will be of diagnostic value rather than mere detection by PCR. The qPCR can also be used to monitor the MD vaccines.

Loop mediated isothermal amplification technique (LAMP): Loop-mediated isothermal amplification (LAMP) technique for rapid detection of MDV meq gene in feathers of infected birds has been developed lately (Wozniakowski et al., 2011; Angamuthu et al., 2012; Wei et al., 2012). LAMP test required 100 fold less copy number for detection of MDV compared to conventional PCR, and the detection time can be less than sixty minutes (Wei et al., 2012). The LAMP technique utilizes three different sets of primers binding to six different sequences thus adding more specificity, the reaction is carried out at isothermal conditions and the products can be visualized by the naked eye (Notomi et al., 2000; Goto et al., 2009).

Use of FTA filter cards for sample collection: The Flinders Technology Associates (FTA®) filter cards are now used in the field for collection, storage and transport of clinical samples for different purposes. The FTA cards are easily available and inexpensive. The FTA cards have been used to quantify Marek’s Disease Virus (MDV) DNA for the diagnosis of Marek’s Disease (MD) and to monitor MD vaccines. Samples of blood, solid tumors, and feather pulp collected in FTA paper have been successfully used for detection and quantitation of MDV genome by qPCR. The results of that study showed that FTA cards are an excellent media to collect, transport and store the samples for MD diagnosis and to monitor MD vaccines.

DIFFERENTIAL DIAGNOSIS

The three grossly similar-looking diseases MD, Lymphoid Leukosis (LL) and reticuloendotheliosis (RE) should be differentially diagnosed. The MDV infection is ubiquitous, but the disease is not. The principal methods to identify the presence of infection are isolation of the MDV virus, demonstration of viral DNA or antigens in tissues, and detection of antibody (Schat and Nair, 2008). Infection by MDV in a flock may be detected by isolating the virus from the tissues of infected chickens. However, the ubiquitous nature of MDV must be taken into consideration and the diagnosis of MD should be based on a combination of MDV isolation or detection of the genome by very sensitive Polymerase Chain Reaction (PCR) and clinical disease (Zelnik, 2004; Schat and Nair, 2008).

Since MDV and ALV viruses are ubiquitous mere detection of their nucleic acid in clinical samples is of limited value unless the virus specific antigen/oncoprotein is also demonstrated in infected/tumour cells by immunohistochemistry. qPCR technique can be used effectively to detect and quantitate the viral copy numbers. Exclusion of ALV and REV when others signs of MD are present will have diagnostic value. Ruling out other disease may help rather than ruling in because of the omnipresent nature of MDV and ALV. As far as REV is concerned detection of viral nucleic acids in tumours is of diagnostic value since the virus is not ubiquitous. The use of B or T cell marker monoclonal antibodies for immunophenotyping of tumour cells is not that useful in differential diagnosis since bursal B cell tumour can be caused by both ALV and REV, and non-bursal tumours caused by REV are CD8 positive T cells.

PREVENTION AND CONTROL

Control of the disease can be achieved by adapting good management practices and vaccination. Chicks should be reared in isolation for 2-3 months from older stocks. Removal of used litter and disinfection of buildings are important to control the disease. Strict bio-security is to be followed to avoid introduction of MDV in the flock. There is no treatment exists for Marek’s disease. Vaccination has been the main approach for its prevention and control throughout the world. Vaccinal immunity, once acquired, is apparently lifelong. However, while vaccination will prevent clinical disease and reduces amount of infective virus shedding thus reducing horizontal disease spread, it will not prevent infection/transmission of the virus. Good management practices along with emphasis on all-in/all-out production, improved sanitation and hygiene, strict biosecurity measures and exploring genetic approaches for increasing the genetic resistance of birds are valuable measures for controlling MD. Genetic selection strategies for MHC have provided with chicken breeds/strains that are MD-resistant (B21) and MD-susceptible (B19).
MDV vaccination: past, present and future: MD vaccines are highly effective, often achieving more than 90% protection under commercial conditions. Herpes virus of turkey strain FC-126 is widely used and is highly effective against virulent MD virus. A bivalent vaccine consisting of HVT and a serotype-2 strain SB-1 have been found to have synergistic effect and provide better protection against virulent MDV. In-ovo vaccination is the method of choice due to no requirement of chick handling. Immunity develops within two weeks. Because vaccination does not prevent infection with the virus, the MDV has evolved with increased virulence and resistance to this vaccine. As a result, current vaccines used are a combination of vaccines using HVT and gallid herpesvirus type 3 or attenuated MDV strain, CVI988/Rispens.

The first Marek’s disease vaccine was described by Churchill et al. (1969) shortly after identification of causative of MD, a cell-associated herpesvirus (Churchill and Biggs, 1967). This vaccine was based on the oncogenic HPRS-16 strain of serotype 1 MDV (MDV-1) that had been attenuated by serial passages using chicken kidney cell cultures. It was licensed in the UK in 1970, but was soon replaced by a new vaccine, based on herpes virus of turkey (HVT; strain FC-126) (Okazaki et al., 1970, Witter et al., 1970). The widespread vaccination provided the host with another important weapon against the virus and the losses from the disease decreased dramatically by over 99% (Witter, 2001). HVT-based vaccines are still widely used, either alone or in association with other vaccine serotypes. In India, Serotype 2 (SB-1) and 3 (HVT) vaccines are still in use (Morrow and Fehler, 2004). An attenuated MDV strain, CVI988, is considered to be the most protective vaccine currently available and has been introduced in many countries (Davison and Nair, 2004). MDV vaccines are administered in ovo at Embryonation Day (ED) 18. The automatic injectors deposit the vaccine inoculum into the amniotic fluid of the majority of the eggs. However, with increase in cases of vaccination failure and the emergence of more virulent pathotypes, the disease poses a severe threat to the poultry industry and challenge the control strategies (Venagopal et al., 2000). Though MD vaccines have efficacy more than 95% (Witter, 1998), they have many inherent drawbacks (Nair, 2005). The most important one is their inability to induce a ‘sterile immunity’ in the vaccinated host. This allows the virulent virus strains to replicate and be shed into the environment in spite of the vaccination status of the host. CVI988/Rispens (attenuated MDV 1) vaccine is useful in turkeys against MD, but HVT was not protective against MD in this species (Schat and Nair, 2008).

Recently, it was found that mcg oncogene deleted recombinant vvMDV strain (rMcDSÃ‡Meq) could not induce tumours in infected chickens confirming that Mcq oncoprotein is essential for transformation of lymphocytes (Lupiani et al., 2004). Two different experiments showed that rMd5Ã‡Meq virus was fully attenuated after deletion of mcq gene and the Mcq-null virus provided protection equal or superior to CVI988/Rispens (attenuated MDV 1), the most efficacious vaccine presently available against MD, following challenge with very virulent (rMd5) and very virulent plus (648A) MDV strains (Chang et al., 2011; Li et al., 2011). Further research is needed to utilize them for field conditions. Molecularly defined recombinant MDV vaccines eliciting sterile immunity will be the future as far as MDV vaccine research is concerned.

CONCLUSION AND FUTURE PERSPECTIVES

Marek’s disease is a disease of chickens produced by a herpesvirus that produces a reduction in the immune response in acutely infected birds followed by the production of tumours in many of the infected birds. Very virulent strains (vvMDV+) have been reported in a number of countries around the world and have affected broilers, breeders and commercial layers. This disease extensively limits the productivity of both egg producing and meat producing birds resulting in great economic impact in poultry industry. Vaccination, in conjunction with good farm cleaning and disinfection, proper reception practices, adequate downtime between flocks, all-in/all-out policy, accurate vaccination programs adapted to the type of birds and field situation, good vaccine preparation and administration practices and strict biosecurity measures can greatly reduce the incidence of Marek’s disease and thereby prevent the economic losses due to the disease. In problematic areas with monovalent vaccine, bivalent or polyvalent vaccine is recommended for the effective prevention of virulent MDV. However, vaccine failures do occur as field strains continue to evolve towards pathotypes of greater virulence. The constant evolution of MDV has pressed us for the development of new vaccines or vaccine strategies that control the more virulent emerging strains. However, the competition between the development of vaccines and evolution of MDV is a major threat for poultry industry.

REFERENCES


