

## Marek's Disease Control in Broiler Breeders

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### Introduction

Marek's Disease Virus (MDV), a highly infectious and easily transmitted herpesvirus, is one of the most common viral infections worldwide and is capable of causing tumors and immunosuppression in broiler breeders. These tumors can be found in different organs of the bird, as well as in nervous, skin, and eye tissue. Other viruses capable of causing tumors in parent stock are Avian Leukosis Virus (ALV) and Reticuloendoteliosis Virus (REV), which are retroviruses that can sporadically cause mixed infections with MDV, complicating their diagnosis. Occasionally tumor lesions unrelated to viral diseases can occur spontaneously in adult broiler breeder flocks.

MDV continues to be a major threat for the poultry industry although in most areas it remains under control by a combination of vaccination and biosecurity practices to reduce the risk of early exposure.

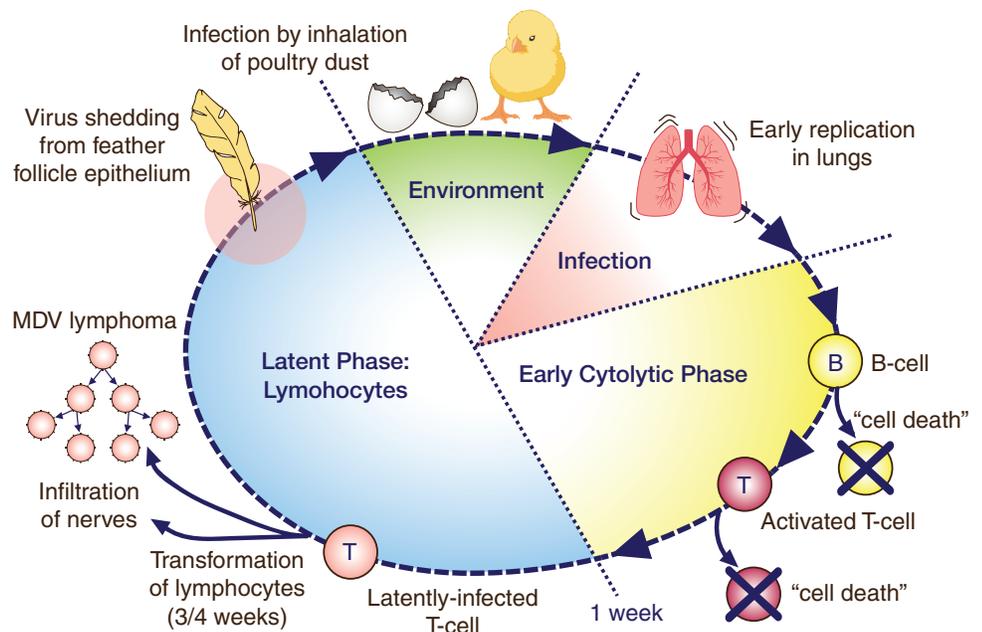
### Infection and Transmission

MDV is highly contagious and easily transmitted from bird to bird. It replicates in the feather follicles, where it is released into the environment through dead cells of infected feather dander and persists for long periods of time. Infections occur when chicks inhale infected dander or contaminated dust and the virus reaches the lungs where respiratory tissue and blood cells (B and T type lymphocytes) become infected. B lymphocytes are responsible for humoral (antibody mediated) immunity while T lymphocytes are involved in cellular immune responses (direct defense against invading organisms).

Eventually the virus reaches the feather follicles completing its replication cycle (**Figure 1**). Effective vaccination prevents the development of tumors from latently (inactive or dormant state) infected T Lymphocytes, and although **infection and shedding of MDV can be reduced, it cannot be fully prevented by vaccination**. Infected chickens become healthy carriers for life and a source of infection to younger birds.

**Figure 1:** Stages of the MDV life cycle.

**Schematic diagram showing the different stages of MDV pathogenesis including the virus shedding from the feather follicle epithelium and the transformation of T lymphocytes in susceptible birds.**



### MDV Vaccines

Vaccination has been the foundation in the protection against MDV. The majority of MDV vaccines are cell-associated, meaning the virus is present in the cells of cell cultures, grown under laboratory conditions, and used to manufacture the vaccine. They are administered in-ovo at egg transfer (18 days of incubation) and/or at hatch by subcutaneous or intra-muscular injection.

Commercially available vaccines include the following:

1. Traditional (live virus)
  - Serotype 1 - [CVI -988 (Rispens)]
  - Serotype 2 - (SB-1)
  - Serotype 3 - [Herpesvirus of Turkeys (HVT)]
2. Viral-vectored recombinant HVT with gene inserts for other viruses
  - rHVT - IBD
  - rHVT - ND
  - rHVT - ILT
  - rHVT - AI

HVT vaccines are used in combination with serotypes 1 (Rispens) and/or 2 (SB-1) to maximize protection in breeder flocks. Today, HVT combined with Rispens is the most common combination used to protect breeders in very high-risk areas.

Viral-vectored recombinant vaccines are made from viruses that have been genetically modified by using a recombinant HVT (rHVT) as a vector for gene inserts (coding for immunogenic antigens) for other viruses. Examples of gene inserts that are used with rHVT vaccines include Infectious Bursal Disease (IBD), Newcastle Disease (ND), Infectious Laryngo-tracheitis (ILT), and Avian Influenza (AI). The advantage of recombinant vaccines is that they generate an immune response against both the vector and the insertion of another virus. These rHVT vaccines should not be mixed with conventional HVT or among themselves because the response to MDV and the corresponding insert will be negatively affected. Likewise, if HVT is used in-ovo, then rHVT cannot be used at hatch because it will result in interference against the recombinant product. Also, it is imperative to be cautious in combining MDV vaccines with other kinds of live vaccines or additives (i.e. antibiotics, supplements) unless specifically advised by the vaccine manufacturer. ***It is important to always follow the manufacturer's recommendations when administering vaccines.***

### Vaccine Handling and Preparation

Most MDV vaccines are frozen in liquid nitrogen. Freeze-dried vaccine (HVT strain only) is available for small flocks or used in countries where liquid nitrogen is not available. Cell-associated vaccines should only be stored in specialized liquid nitrogen containers (-196°C/-320°F), reconstituted in a specifically supplied diluent, and administered following precise procedures provided by the manufacturer. Preventing bacterial contamination during vaccine preparation and administration is key when preparing the MDV vaccine. Proper mixing and hygiene procedures in the hatchery's vaccine preparation room and for the vaccination equipment must also follow the manufacturers' recommendations at all times.

Vaccine storage, thawing, reconstitution, hygiene, and administration procedures require regular training of hatchery personnel and continuous auditing by internal (quarterly) and external (annually) quality control specialists.

### Administering MDV Vaccines

The administration of MDV vaccines in-ovo results in better protection against early MDV challenges and has a positive effect on the chicks' immune system and responses against other non-related antigens (molecules that induce immune responses). Also, administration of MDV vaccine in-ovo followed by a second dose at hatch can improve the protection against early challenge with very pathogenic strains in field situations, such as areas with a heavy concentration of poultry farms, multiple age farms, use of re-used litter, and breeder chicks traveling long distances after hatch.

Reconstituted vaccines must be maintained under refrigeration while being administered and used within 30-60 minutes. MDV vaccines are unstable suspensions of cells, and therefore, proper mixing and periodic shaking will prevent sedimentation and ensure a uniform dose of administration.

Further information on vaccine strains, vaccination methods and vaccination regimens for breeders can be found in the **Aviagen Marek's Disease booklet (2017)** or by consulting with the Aviagen's global veterinary services department. Selecting the most protective vaccine strain, vaccine combinations and administration methods is critical to maximize the protection against the disease and associated bird mortality.

### Causes of MD Outbreaks

After vaccination in the hatchery, chicks are not protected until the MDV vaccine strain multiplies in the individual chicks and starts circulating in the blood (known as viremia), which can take between 4-5 days. Therefore, it is essential to reduce the risk of early environmental exposure to MDV and/or delay the time of infection for as long as possible so the birds are fully protected. This is best achieved by placing chicks on new litter and a single age, biosecure farm. Built-up litter and multiple age rearing farms pose a very high risk of early exposure to field MDV and other immunosuppressive viruses. Proper selection and administration of MDV vaccines will result in adequate control; however, MDV breaks could occur as a result of the following factors (alone or in combination):

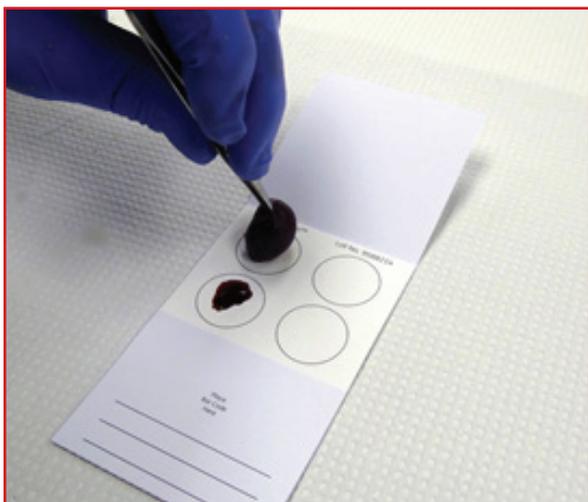
1. Inadequate vaccine storage, handling, preparation or administration procedures.
2. Suboptimal doses or dilution of vaccines.
3. Additives (antibiotics) that change the pH and/or other properties of the diluent.
4. Interference to MDV vaccination response caused by other vaccines.
5. Early exposure to very virulent (vv) or very virulent + (vv+) MDV strains.
6. Immunosuppression caused by:
  - Infectious factors:
    - « Infectious Bursal Disease Virus (IBDV)
    - « Infectious Chicken Anemia Virus (ICAV)
  - Flock management factors:
    - « Overheating during hatch, processing and transport
    - « Poor brooding conditions (incorrect house environmental conditions, inadequate provision of feed and water)
    - « Extreme heat and cold (environmental temperatures), inadequate ventilation
    - « High stocking densities, poor feed distribution, inadequate feeder space, poor body-weight development
  - Nutritional factors:
    - « Poor ingredient quality and mycotoxins
    - « Suboptimal levels of essential nutrients

### Diagnosis

Tumor like lesions can develop in birds as young as three weeks of age and typically are most common before flocks reach sexual maturity. However, tumor lesions can also be found during post-mortem examinations of flocks around peak egg production (late MDV). Since essentially all healthy birds are carriers of MDV without showing symptoms, virus isolation from blood samples does not have any diagnostic value. Diagnosis is based on typical gross lesions such as enlarged peripheral nerves, tumors in various internal organs, nodules in the feathered skin and grey, irregular eyes. It is confirmed by histological examination of a complete set of tissues (see list below) by a trained poultry pathologist. Furthermore, a conclusive diagnosis can be achieved by histochemistry or the presence of high loads of Marek's viral DNA by polymerase chain reaction (PCR) testing. Detection of large quantities of viral DNA by real-time PCR in blood, tumor cells, and feather pulp provides a specific diagnosis of MDV even in mixed infections with other viral tumor induced diseases. A summary of required specimens and diagnostic procedures is presented below:

- Histopathology (complete set of tissues placed in 10% buffered formalin):
  - « Liver, kidney, spleen, proventriculus
  - « Peripheral nerves (sciatic nerve), brain
  - « Bursa, skin, and eyes
- PCR tests and histochemistry
  - « Frozen tissue
  - « FTA cards (imprints of tumoral tissue) (**Figure 2**)
  - « Paraffin embedded fixed tissue

**Figure 2:** Example of tumoral tissue imprinted onto FTA cards.



Diagnosis must be performed as a multi-step process. When mortality due to tumor lesions is suspected fresh dead, lethargic, and paralyzed birds must be examined at the farm or submitted to a diagnostic laboratory to collect specimens for confirmatory tests as described above. In addition, a complete flock clinical and vaccination history must be collected along with the number (or percentage) of birds with suspected tumors from the total number of birds examined. Once the diagnosis is confirmed it will be necessary to investigate and determine the cause(s). Mixed infections with MDV, ALV, and REV can occur, and therefore, it will be necessary to conduct various tests as histological exams alone cannot be used to make a final diagnosis. Virus isolation is only performed (from peripheral blood white cells, spleen, and tumors) to evaluate the pathogenicity of a field strain.

### Conclusions

- MDV is present in all commercial flocks regardless of vaccination or health status.
- MDV continues to be a threat due to evolving and increasingly pathogenic strains.
- MDV mortality in parent stock is due to tumor lesions and immunosuppression.
- Vaccination does not prevent infections and shedding of the pathogenic field virus.
- Biosecurity helps to reduce risk of early exposure to MDV and other immunosuppressive diseases.
- Cell-associated (frozen) vaccines require careful management, and its preparation and administration require continuous training and auditing.
- Careful selection of appropriate vaccine strains and vaccination methods is critical to maximize protection and done following manufacturer's recommendations.
- MDV outbreaks can be caused by improper vaccine selection, handling, and administration or as a result of early exposure to highly pathogenic MDV and immunosuppression.
- Diagnosis of MDV is a multi-step procedure requiring laboratory confirmation.
- If MDV is confirmed, an investigation must follow to identify contributing causes.

For further information, see the Aviagen booklet - *Marek's Disease Virus* and the Veterinary How To - *Take FTA Card Samples* found at [www.aviagen.com](http://www.aviagen.com).