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I. – Introduction

Marek's disease (MD) is a very infectious lymphoproliferative disease of domestic poultry, causing high mortality and tumor lesions in many visceral tissues with predilection in nerves and skin. MD is still economically very important in poultry production especially in unvaccinated flocks. It occurs mainly in domestic fowl, rarely in turkey and quail.

MD is caused by oncogene Marek's disease virus (MDV) identified as Herpes B virus (16, 40). MDV is ubiquitous and very frequent in almost all poultry populations raised under commercial conditions. It is spread all over the world, including Mediterranean countries. Over 80 years of research on MD has been completed since the Hungarian veterinarian Professor Dr. Josef Marek first described nerve disorders in poultry. He named this paralytic disease Polineuritis gallinarum because of the lesions in many nerves, especially the thickening of sacral plexus with infiltrations by mononuclear cells, and paralysis of the legs and wings. After his work, research continued and the disease was referred to for many years as Marek's paralysis, fowl paralysis, Neurolumphomatosis gallinarum, acute leukosis, etc..

Between the two World Wars many researchers described in detail the clinical and epizootiological features of MD macroscopic and microscopic lesions and transmission experiments. Many authors, although not all, thought that it was infectious but they could not ascertain the agent. The transmission with tumor material or whole blood proved their suspicions but in those years they did not know that the infectious agent was cell-associated. MD was reported in the USA, Germany, England, South Africa, Japan, etc..., mainly as chronic (classical) with moderate mortality.

After the rapid development of poultry production all over the world, accompanied with high density of chickens, MD became very important and has been very intensively researched over the last thirty years. High mortality and low production started in the 1950s. Acute forms of MD were reported in the USA in 1957, England and the Netherlands in 1965, and in other European countries. In Yugoslavia ND occured in one multi-aged broiler farm with a capacity of over 160,000 chickens and its own hatchery. Mortality started in 1961 and increased by the end of 1965 when the farm was depopulated.

The isolation of MD herpes virus B (MDV) in the late 1960s from tumor cells of sick chickens and its propagation in tissue culture definitively explained the aetiology and pathogenesis of MD). Many pathogenic and nonpathogenic strains of MDV were isolated in chickens. The apathogenic herpes virus was also isolated from healthy turkeys (HVT). This virus is antigenically related to MDV and is present in most turkey populations, but it does not occur in chickens.

Soon after these isolations, some strains of MDV were attenuated through serial passage in tissue culture of chicks kidney cells, and prepared as cell-associated vaccine. HTV was prepared as cell-associated, and especially cell-free vaccine, which was widely used all over the world.

With this very successful work, which was accomplished by a number of scientists mostly in Great Britain and the USA, MD was finally distinguished from lymphoid leukosis, which was for many years confused as

a form of Avian leukosis complex. In recognition of Dr. Marek's work, WVPA (1960) proposed the term Marek's disease.

Recently, some very useful reports have been published on the immune response to MD, genetic resistance and control of MD. This paper will present only some facts concerning the practical control of MD.

II. – Spread of the infection

MDV spreads rapidly throughout flocks by direct and indirect contact with infected chickens, premises, litter, dust and chopped feathers. Most important is the airborne route of infection. Very soon after infection of the respiratory tract, cell-associated viremia can be detected in the blood, reaching a peak about eight days later. Macrophages carry and distribute MDV all over the body infecting sensitive cells and causing lymphocyte transformations. At this time cytolisis and atrophy can be observed in the bursa of Fabricius. MDV may be detected in epithelial cells of feather follicules five days after infection causing degeneration of feather follicules or neoplastic lymphoproliferative lesions in tissue around them. Viral antigen, intranuclear inclusion bodies and cell-free virus can be detected in the skin, on the only sites where cell-free virus is formed.

Sheddings of the virus from the skin through desquamated dander, start before the clinical signs of disease, and may continue throughout the whole life, but with age the virus sheddings lose pathogenicity.

MDV in danders and feather follicules survive more than one year in dust, walls, fans and probably in litter depending on outside temperature, and are the main source of infection. Dust with danders and moulted feathers can be readily transported passively from infected houses over long distances by air or with clothing, infected eggs, utensils, trucks and other ways.

Some ground arthropods were shown to be passive carries of MDV but they are not a major vector in modern houses. The transmission of MDV by mosquitoes, mites and oocysts of coccidia has not been proven. Chicks can usually be infected very early in life, from the first day after hatching, although it may be some weeks or months later. With age the possibility of infection is reduced because of increased resistance.

Up to now vertical transmission of the virus has not been proven. Day-old chicks are free of the virus, although they could be infected in hatcheries which had not been disinfected.

III. – Viruses

The incidence of MD depends of the pathogenicity of MDV. A number of MDV strains, serologically related but distinct groups, were isolated from sick chicks. Currently they are classified into serotype 1 and serotype 2 groups, while herpes virus isolated from healthy turkeys (HVT) is classified as serotype 3. This classification is based on the indirect immunofluorescence test, the agar-gel precipitation test, and virus-neutralization assays. Virus isolates vary in their pathogenicity to chickens. All strains from serotype 1 are pathogenically different, strains from serotype 2 and 3 are apathogenics.

Some of these isolates can cause a high incidence of MD ; some were prepared as useful commercial vaccines (**Table 1**).

On most farms chicks infected with some of these viruses can be found. The incidence varies and it depends mainly on the oncogenicity of MDV, genetic resistance, environments, age, material immunity of host, management and stress.

Table 1: Strains of MD virus isolates

Origin	Strain	Serotype	Vaccines	Pathogenicity
Chicks	HPRS 14 (2)	1		Moderately pathogenic
	HPRS 17 (32)	1		(classical)
	Coon A (14)	1		"
	CVI 988 (35)	1	att. cell-associated and Clone C	"
Chicks	HPRS 16 (26)	1	att., cell-associated	Highly pathogenic (acute)
	GA (18)	1		
Chicks	MD 11 (41)	1	att., experimental	Very virulent
	RB-1B (37)	1		(variant biotypes)
	Crescenti (29)	1	att., experimental	"
Chicks	HPRS 24 (5)	2		Apathogenic
	SB-1 (37)	2	cell-associated	
Turkeys	FC 126 (40)	3	HVT, cell-associated or	"
	PBI (16)	3	lipphylized	Apathogenic
				"

In the serotype 1 group of MDV, a large differentiation of oncogenecity exists within some isolates. Infection with variant biotype viruses can cause high mortality, more lesions in tissues in genetically resistant, unvaccinated birds or in genetically susceptible and HVT vaccinated chickens. Infection with highly pathogenic MDV, such as HPRS 16, can cause a high incidence of MD in genetically susceptible but not in resistant chicks. Infection with CVI 988 MDV causes minimal lesions only in genetically very susceptible birds.

The incidence of MD on a farm with the same genetic stock and same age differs between houses and even between parts of houses. A flock from a single house, or a part of it, could be infected with high pathogenic MDV and chicks from other houses with viruses of low pathogenicity or even with apathogenic viruses. Chicks can be infected with more than one strain of MDV. The incidence of MD is associated with the predominant type of virus at the time of primary infection. Primary infection with pathogenic viruses predominant after infection had higher outbreaks than infection with apathogenic viruses as predominant. Infection with apathogenic MDV prior to infection with pathogenic strains may induce natural immunity and reduce incidence of MD.

IV. – Immunoprophylaxy

1. Specific control of Marek's Disease

Spread and outbreaks of MD with high condemnations and mortality continued all over the world until 1970-71, when vaccination against MD was introduced. In the late 1960s mortality reached 45 to 80 percent one some farms but was usually 10 - 30 percent. Since the introduction of MD vaccination with live virus vaccines in all broiler breeder and layer flocks and, in many parts of the world in broiler chickens too, the incidence of MD has decreased dramatically. Vaccinations were carried out first with cell-associated vaccines, i.e. attenuated MDV strains HRPS 16, CVI 988 and HVT, and shortly after with highly successfully freeze-dried HVT strains which are still in use in many countries. Despite the reduction in MD mortality and condemnation, achieved through vaccination, the frequency of MD infection remains high.

When vaccination started in many countries, some outbreaks of MD occured in vaccinated flocks because of unproper maintenance of cell-associated vaccines, not enough use of attenuated strains of MDV, poorly disinfected farms, bad isolation of vaccinated chicks, etc...

After the late 1970s and especially after 1980-82 in some countries, the incidence of MD resurged in vaccinated flocks, first on some mismanaged multi-aged broiler and rearing farms. Heavy losses occured at the start of production between 20 and 30 weeks of age. MD was found in completely vaccinated farms with many houses, or only in one house, or even in one part of a single house. Most of these outbreaks were analysed and failures of vaccination were identified in many aspects.

In many cases the possible causes of most vaccination failures included incorrect dose of vaccine, poor storage and handling of live virus vaccine, immunosuppression caused by environmental factors or virus infections, very early challenge of vaccinated chickens by pathogenic MDV before immunity has developed, interference with maternal HVT antibody and poor attention on aseptic work at vaccine administrations.

With the isolation of very pathogenic oncogene strains of MDV (very virulent, variant biotypes) in genetically resistant and HVT vaccinated chicks in the USA and Italy MD has again become very important.

The variant biotype strains of MDV seem to have become widespread. Their presence in Mediterranean and other countries all over the world has been suspected. Heavy losses of up to 40 percent in HVT vaccinated flocks have occurred in recent years in Italy, Spain, Tunisia, Israel and Yugoslavia on some individual farms, but without proof that this very virulent MDV was responsible. Lesions established in these outbreaks were mainly tumors in visceral organs.

In the USA these strains have been more viscerotropic than neurotropic and were not always isolated from immunity breaks. In recent incidences of MD in Yugoslavia, similar lesions were found in HVT vaccinated chicks as tumors in gonads, lungs, liver, heart, proventriculus, skin, bursa of Fabricius and (very rarely) in the nerves.

The occurence of very virulent strains of MDV is theoretically possible because of mutation of some first isolated strains of the MDV. This is proof that all strains of MDV isolated before 1975 were not very virulent, and that oncogenic activity arose by mutation. Their spread was enhanced by inefficient vaccination, bad management and poor hygiene.

As several authors concluded vaccination only with HVT vaccine cannot portect flocks from infection. These facts demand changes in strategy against MD, reconsidering of vaccine programs, improving post-vaccinal treatment, management and hygiene measures, and work on genetic resistance.

2. Control by polyvalent vaccines

Very successful vaccination with single vaccine virus was highly effective for many years, and is still practiced in most countries around the world. After the isolation of very virulent MDV, frequently from incidences of MD in HVT vaccinated flocks or rarely with another monovalent vaccine in the USA and some European countries, poultry producers have called for the development of more effective vaccinations.

Failures of vaccination, and immunity breaks have been reported many times. Established outbreaks of MD in vaccinated flocks were not always connected with infections of variant MDV biotypes. Incidences of MD in such flocks depend on many factors, but mainly on poor immune response of vaccinated chicks. Because of this, vaccination with HVT vaccine was improved by increasing the dose, preventing and delaying early infection after vaccination, avoiding interference with homologous material, antibodies revaccination at 21 days of age (Great Britain), alternate vaccination of parent flocks and progeny with HVT and attenuated MD vaccines (serotype 1), and vaccination of 18 days old embryos. Besides, improvement was carried out by reducing errors with the application of vaccine, such as correct dose, giving attention to intramuscular or subcutaneous routes of administration, frequent check of calibration of hand-operated automatic syringes or machines, proper reconstitution of vaccine in diluent (especially of

cell-associated vaccines), slow and attentive work of operators, and avoiding mixing some atibiotics with vaccine, which have destructive effects on vaccines.

All these measures had little effect if very virulent virus infected genetically susceptible vaccinated chickens. The most useful method currently in practice is the use of bivalent or polyvalent vaccine containing different serotype of MDV on farms where the presence of highly oncogenic virus is assumed.

However, it is not necessary to use polyvalent vaccines on farms where very pathogenic strains of MDV do not exist as is the case on strongly isolated breeding farms in some districts of Yugoslavia and other countries. With the use of HVT vaccine only we achieved excellent immunity, and we were free of disease on several farms for many years. But incidences of MD in vaccinated flocks with very heavy losses in the same areas demand changes in vaccination policy.

For improving immunity, especially against very highly pathogenic MDV isolates, various virus vaccines containing viruses from serotype 1 and serotype 2 MDV, combined with HVT, have been assayed in some laboratories and field trials, based on synergism between viruses.

In the USA, where very virulent MDV was first established in areas with a high density of chickens, after laboratory and field trials with vaccinal strains serotypes 1, 2 and 3 (MD 11 , SB-1 and HVT), it was found that protection against very virulent viruses was more effective than with any other monovalent vaccine, and without interferences with antibodies. Vaccination with two vaccinal strains from serotypes group 2 and 3 showed better results than vaccination with vaccine from serotype 3 alone. In 1983 bivalent vaccine containing heterologus HVT strain and homologous SB-1 apathogenic strain was licenced. This vaccine was used in areas where incidences of MD occurred, and could protect against highly oncogenic strains under field conditions.

Similar results were achieved in Italy, Spain, Israel and Germany, when vaccinating with various polyvalent vaccines combining HVT with attenuated strains (serotype 1) and apathogenic (serotype 2) vaccines. Very satisfactory results were achieved in Italy with bivalent vaccine containing HVT strains and CVI 988 on certain problematic farms.

More recently, after isolations of very virulent strains in Italy, a bivalent vaccine containing attenuated HPRS 16 and HVT strain was licenced for use.

The selection of a combination of vaccines should be done in connection with the epizootiological situation on farms or in regions. In Yugoslavia we vaccinated about two million broiler breeders with single CVI 988 vaccine because heavy losses on some farms vaccinated with HVT vaccine. Incidences on these farms were lower after vaccination than in previous flocks, but on all these farms management and hygiene were improved. On a couple of vaccinated farms, where isolation and hygiene services were bad, MD re-appeared.

In a multi-aged broiler breeder farm, we experimentally vaccinated chickens from two houses out of eight with a combination of CVI 988 and lyophilized HVT vaccines while other houses were vaccinated only with HVT vaccine. Up to now we have better protection in houses vaccinated with both vaccines than in other houses.

As we know, many flocks in Mediterranean countries were recently vaccinated with bivalent vaccines with beneficial results. We have, however, to keep in mind that vaccinations with all kinds if vaccines cannot be a substitution for errors at vaccinations, bad management and poor hygiene.

V. – Other ways of controlling Marek's Disease

1. Control by management and hygiene

Controlling MD by vaccination is not the only method. Before introduction of vaccination in poultry production, management and hygiene were the only means to control it. After evident success in protection by MD vaccine, basic principles of good managements and hygiene have frequently been forgotten. For many years vaccination has been a preferred control procedure and was obligatory worldwide for broiler breeders and layer chicks.

With bad management and hygiene it is impossible to achieve good protection even using all kinds of available vaccines. Very early infection with pathogenic MDV compromises all initial effects of vaccination. Aerogene infection in the first days after vaccination of day-old chickens is possible in all houses and farms with weak veterinary and hygiene services. MDV is present in the dust of many farms and their surroundings through desquamation of feather follicle, epithelian cells and danders. Infected dust sticks in the environment on walls, litter, feeders, drinkers, ventilation systems and fans. MDV can survive in infected houses for more than one year.

Rearing chickens in isolators with controlled management is impossible for commercial poultry production. Management and hygiene measures for such production should be improved and controlled as follows :

- ☐ vaccinated day-old chicks have to be housed in clean, disinfected commercial houses, and have to be protected through strict isolation for at least 2 - 3 weeks ;
- ☐ cleaning of houses requires mechanical removal of old litter, all dust and organic matter from all surfaces prior to final disinfection. Washing of houses should be done with detergents and high pressure apparatus ;
- ☐ all movable equipment should be cleaned outside the houses, and if possible, fans too, especially from inside ;
- ☐ satisfactory disinfection should be done by formaldehyde vapour, liquid fromalin, chlorine, organic iodine, cresylic acid, or quartenary ammonium compounds ;
- ☐ old litter has to be deposited far from poultry houses ;
- ☐ young chickens should be reared far away from old flocks. One farm should be used for only chickens of the same age. In the very big broiler farms such measures are often impossible. Such farms should be filled with chickens, which are no more than 10 days different in age ;
- ☐ spread of MDV may be restricted with an "all in - all out" policy with complete depopulation of the farm prior to its cleaning out ;
- ☐ the broiler and rearing farms have to be empty after production for 2 - 4 weeks. Broiler farms can usually be refilled very soon but not before 2 weeks ;
- ☐ good insecticide should be applied as it is known that some beetles can carry viruses from one flock to the next ;
- ☐ hatchery, slaughter houses and farms have to be far from one another to avoid infection of day-old chicks. Raising chicks on the backyard of individual farms, even neighbours, is not allowed ;
- ☐ all employees and professional visitors have to wear overalls, clothes, boots and head covers for each farm separately and, on the breeding farms, should even change underwear.

- ☐ disinfected foot pads have to be renewed daily, especially in the winter ;
- ☐ if possible, chickens should be held in the hatchery for one day after vaccination to avoid very early infection ;
- ☐ avoid stress of chickens in transporting from hatchery and stress in houses from the first day. Optimal brooding temperature, clean but not cold water and good feed help to prevent predisposition to infection in chickens.

2. Control by breeding for resistance

Genetic selection for MD resistance together with other control methods, should be most important and of great value for poultry breeders and poultry production. For more than 50 years it is known that in many breeds genetically controlled differences exist in susceptibility to MD. Now it is evident that breeders have to pay more attention for continuing work in genetic control of such resistance in their breeds.

A study of genetic resistance for lymphoid leukosis has given very good and practical results, but such a study has not been done for MD. Nearly all poultry breeders stopped or reduced support for this research. Satisfaction with very good results of vaccination against MD over many years was the main reason for this stagnation.

The isolation of very virulent variant MDV in susceptible and HVT vaccinated flocks increases the need for such study. Besides, vaccinal immunity was greater in resistant than in susceptible flock. Vaccination with HVT vaccine protects resistant chickens against very virulent pathotype MDV but not susceptible chickens. Infection with these viruses cannot be prevented with vaccination or genetic resistance alone, but the combination of both prevents high losses from MD. For this reason, resistance to MD is, or should be, included again as one of the traits considered in genetic selection by poultry breeders in spite of the possibility that selection for resistance to MD may show negative correlations with other commercial traits such as hatchability or growth rates.

Methods for selction for MD resistance are different and have been reported several times. Many breeders select chickens on a simple random basis, breeding survivors from the field infection or with controlled challenge with pathogene viruses. The choice of virus for challenge infection is critical. Virus with low virulence does not induce high enough incidence of MD to provide a basis for selecting survivors, while viruses with high virulence can induce MD in a great number of genetic resistant chicken.

A more scientific method for detecting genes for resistance to MD is the blood group technique. The genetic resistance to MD has been found to be controlled by two distinct genetic loci. Resistance to MD is associated with genes at the erythrocyte antigens of blood group B locus.

Blood group typing can detect those individuals homozygote for the particular allele conferring resistance. Chickens with B^{21} blood group allele were found to be more resistant to MD than those with B^{19} allele. Other alleles as B^{2} or B^{6} confer some resistance too. The B^{21} allele is widely distributed in poultry populations, suggesting that it has survival value for the species. Differences in susceptibility is associated with some other B alleles but B^{21} linked resistance to MD is inherited as a dominant trait. This resistance may result from better immunological responsiveness.

This genetically determined resistance to MD in commercial breeds should be of great value, since it is known that HVT vaccine is excellent and efficacious in protecting resistant chickens.

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