Marek's Disease: History, actual and future perspectives

Dr Frank Fehler (Cuxhaven, Germany)

The causative agent in view of evolution and history

The Herpesviruses belong to the virus families most wide spread in vertebrates. If any vertebrate species is investigated intensely enough at some point a member of this virus family will be found. It seems, that this virus family developed together with it's hosts. The fact, that in large number of cases the virus must be searched for, demonstrates, that many members of this family do not induce any clinical manifestations in their host. This is of interest to the virus since the life supporting system should not be damaged severely in order to ensure it's own survival. Most representatives of the Herpesviridae therefore behave as classical commensals.

However, some of the Herpesviruses have evolved into pathogens which cause severe problems in their hosts. The development of such pathogens is often linked to the presence of large host populations with high densities or to exchange of individuals (or material) between populations. These conditions enable the virus to spread rapidly. It starts to adopt to the situation. The possibility of rapid spreading pushes potential damages of the single host into the background. Efficient and fast replication starts to be a selective advantage.

The development of a pathogenic virus is thought to follow the described or a related way. In fact, many diseases do occur at a point where suitable environmental conditions are created. Thus at the beginning of the 20th century, accompanied by intensification of the poultry economy, a new pathogen appeared, which as all Herpesviruses is difficult to control until today. It caused the Marek's disease.

High animal densities, genetic monoculture (created for high performance), intensive exchange of breeding material and little knowledge of hygiene made their contribution to this triumphant progress. Herpesviruses in general exhibit a tremendous adaptability to changes in their environment. They can escape from selective pressures by mutations. An exceptional capability of these viruses is the uptake of genes from the host organism and the use for own purposes. One or several of these adaptation mechanisms may have led to the appearance of oncogenic properties of the Marek's disease virus (MDV). This oncogenicity is the main problem of the disease today and causes tremendous economical losses. In later steps of disease development neurological signs (transient paralysis) and skin lesions occurred in addition to lymphomas.

In the early 1920's outbreaks with high mortality and high incidence of visceral tumors already occurred. The environmental conditions described above favoured a rapid spread and the disease could soon be found all over the world.

First measures to fight the disease

Early attempts to become rid of the disease involved breeding for genetic resistance starting from the mid of the 30's until the late 60's. These attempts , which are still going on, demonstrated that genetic resistance is linked to certain genes of the major histocompatibility locus of the chicken. Later and more detailed results showed that a number of additional genes have important function in control of Marek's resistance. Today 14 genetic loci linked to genetic resistance against MDV are identified on 5 chromosomes of the chicken genome. This makes the breeding for highly resistant animals much more complicated. The classical breeding method for highly resistant animals may lead to positive selection of genetically linked characteristics, which may be detrimental to the goal of achieving high performance in these birds.

Another problematic aspect of these breeding programmes that should be addressed is the establishment of genetic monoculture mentioned above. Uniform genetic populations may lead to an adaptation of the pathogen to the new genetic environment conditions. In this case the breeders would not be able to react fast enough to beat the constantly evolving pathogen. In another scenario a totally different pathogen may find ideal conditions within the new genetic environment and therefore may spread rapidly causing tremendous losses.

Vaccines

Since the beginning of the 60's vaccination forms the solid basis of fighting Marek's disease. At that time the first Marek's disease virus was attenuated by continuous tissue culture passaging.

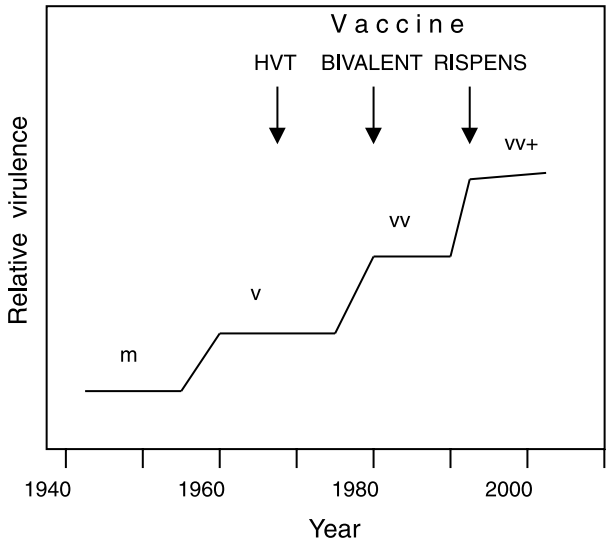
The first vaccine, which has been extensively used in wide geographical areas was a Herpesvirus, which was isolated from turkeys (HVT, serotype 3). Later attenuated strains of serotype 1 and 2 followed. The finding, that vaccination against an oncogenic viral disease is possible was a totally new concept (the possibility of vaccination against such a disease is still unique). For a long time this fact has been the reason for using the chicken as a model for oncogenic viruses in man. This led to some progress in Marek's research but a profit for human medicine can not be deduced at this point in time. The possibility to have such a vaccine is very fortuitous for the poultry economy.

Unfortunately the use of vaccines did have negative aspects too. About 10 years after the first extensive usage of the HVT vaccine, field viruses started to adapt by unknown mechanisms. Despite vaccination, more and more Marek's cases occurred. Luckily at that time a new vaccine strain of serotype 1 was found. It is still known under the name of the scientist who isolated it, Dr RISPENS. With this strain the escalating situation could be controlled rapidly. After a second decade however, an increasing number of vaccine breaks occurred again which could explained only by adaptation of the field virus to the selective pressure caused by vaccination. In Europe a combined vaccination of both serotypes (1 and 3) was chosen to deal with this emerging disease. With this bivalent vaccination they were successful again to curtail the disease.

Depending on the local field situation today different countries are using one of the monovalent or bivalent vaccines. In the United States the situation became more complicated since an additional serotype has been used in monovalent or within combined vaccines (up to trivalent). An overview on the development of the American situation (according to WITTER, 1997) starting from the 40's is given in Figure 1. The use of new vaccines with increasing

potency seems to correlate with a selection in the field for new variants which exhibited higher pathogenicity. Looking at this type of figures it should be taken into account that these correlation schemes neglect the fact that during this long period the genetic background of the animals changed dramatically.

Figure 1: Evolution of Marek's disease in the USA



The use of all the vaccines described above resulted in a decrease in occurrence of Marek's disease by 99 % during the last 30 years. However, a complete eradication of the disease could not be achieved.

The actual situation

Today, another decade has passed since the last big wave of infection and again a rumour is rising in the poultry world that another more pathogenic MDV is coming. REDDY et al. (1997) demonstrated in the United States the appearance of new highly virulent strains (so called vv^+-strains) which broke the protection induced by bivalent vaccines.

At this point it should be mentioned that the vaccine strains used in the US are more attenuated as in Europe. Additionally it became clear that US veterinarians relied too much on vaccination and neglected the valuable means of proper hygiene and good flock management. However, after recognising the reasons and after taking suitable measures the situation could be controlled in so far as at least the most severe losses could be avoided. Today the vv^+ viruses seem to be limited to the area, where they have been described first. They are kept under control by use of conventional vaccines and intelligent hygienic management.

Despite these facts the American incidents shook the poultry industry out of it's apathy and increasing world wide attention was given again to Marek's disease. In many cases the appearance of increased mortality and tumors was interpreted as vaccine failure or a break of immune response by highly virulent viruses.

The true reasons in most cases can not be reconstructed when the disease is already spread within a flock. A survey carried out at the last international congress on Marek's disease in Montreal (August 2000) asking responsible veterinarians from more than 25 countries gave the following results: in none of the countries a striking increase in frequency and severity of the disease occurred. All participants were in agreement that the means for fighting the disease are not perfect but sufficient to control the actual disease situation. The diagnostic means were assessed as sufficient despite the fact, that Marek's diagnostics still need a long experience to do it in reliable manner.

Marek's disease as a multifactorial problem

How does the actual field situation appear in reality?

To give an overview on the overall situation seems to be impossible, since there is no obligation to notify the authorities of disease outbreaks. In addition the disease is a very sensitive problem in the field since many poultry producers do not like to talk about it. Thus it can occur that responsible veterinarians from the same country paint a totally different picture of field situation.

However, in summary it can be said that there are still many problems in the field with Marek's disease. These may differ from country to country. The countries in the south of Europe (like Spain) have often severe problems in meat type birds, while others like France and Germany struggle currently with increased Marek's mortality in layers. Some countries have general problems in all segments of intensive poultry management.

What are the causes of these outbreaks?

The list of possible causes is long. At this point I want to state in advance that until today nobody was able to show the occurrence of vv^+ strains according to the definition of WITTER (break of bivalent vaccinations in high percentage) outside of the North American continent. In many cases other reasons than the high virulence of the field virus are responsible for the outbreaks of disease.

1. The main reason for the occurrence of increased mortality is the **improper handling of the vaccines** used currently. Marek's vaccines are the most sensitive vaccines one can imagine. This is especially true for the widely used cell-associated vaccines. They must be kept at -196 °C during transport and storage until use. Even the thawing of the ampoules at temperature of more than 28 °C or a long incubation time after dilution may cause damage to or destruction of the vaccine virus.

   Especially in developing countries improper handling often causes problems. In these areas difficult transportation routes, hot climates and low education of the vaccination personnel are additional reasons. But not only heat and improper storage are problematic. Often more vaccine is diluted than can be administered within the first two hours. In some cases the vaccine from on supplier is diluted with the diluent of another one or the vaccine is mixed with that of other suppliers, different vaccines (which means against other diseases) or with antibiotics.

   This problematic situation is exacerbated by the fact, that there is no real proof for successful vaccination. But even if there would be a control in many cases it would be too late to avoid a field infection.

2. **Hygiene** is a second important point in fighting Marek's disease. The Herpesvirus is spreading mainly in dust particles which are shed from the feather follicles of

infected animals. If young birds are introduced into houses, where infected birds have been kept before a maximum of hygiene should be kept. A lack of good hygiene within the houses may have been one of the main reasons for the development of the situation in the US since a disinfection of the houses between two rearing periods following one another was usually not carried out.

At this point one should be aware of the fact, that a Marek's vaccination does not lead to a sterilising immunity. This means the animals are protected from symptoms of disease but not from infection. The shedding of the virus by feather follicles is often reduced but still present. Pathogenic viruses are still released from feather follicles despite vaccination and threaten unvaccinated chicks at the same location. The stability of such viruses in dry dust can be more than one year. The reason for this is not yet clear. One explanation is the occurrence of free virus, that seems to occur only in feather follicles. This kind of cell free virus would be more stable. Another explanation discussed is the enwrapping of the virus into ceratinised particles that protect the virus from destruction.

3. The preceding explanations make clear how important good **flock management** is to control the disease. Especially in locations where chickens of different age are kept dramatic losses may occur. The old animals at the production site are vaccinated and show no clinical signs due to MDV. However, they can be shedders of field virus. When young animals (especially during the first week of life) are housed they are still susceptible for Marek infections from dust since the vaccination which as been carried out at day old did not yet induce a full protection. A combat between vaccine virus and field virus within the host starts. An increased mortality may occur that may build up in the next generations. These procedures have been carried out in America recently. In contrast most European chicken integrators are working on according the "all in - all out"- principle and by that means they reduce the reservoir of the field virus since all animals within a flock are developing immunity nearly at the same time. Together with a proper hygiene before and after each generation housed the infection risk is minimised. However there are still some residual problems with the disease.

4. A mostly unknown aspect of Marek's disease outbreaks is the **influence of other pathogens** which can cause immunosuppressive effects in the host. Examples for such pathogens are the infectious bursitis virus (IBD), the avian leucosis virus (ALV), Reoviruses and the chicken anaemia virus (CAV). Especially the latter becomes more significant in many regions within and outside Europe. During the last year many cases of Marek's disease were investigated as part of an EU framework project on development of a next generation vaccine against MDV. In 11 of 15 cases (from 8 countries), from which proper material from acutely affected animals was available, CAV could be shown as coinfecting virus together with the MDV. The clinical signs accompanying this coinfection occurs predominantly in young layers before or directly at point of lay. The MDV specific mortality developed normally in these flocks until a certain point. Then morbidity and mortality increases abruptly. Transient paralysis in these flocks is rare. Often a dramatic weight loss occurs. The frequency of tumors in sick and dead animals is relatively low, but classical symptoms like swelling of the proventriculus are present.

   This novel phenomenon presumably can be traced back to the immunosuppressive effects of CAV. Normally it is believed, that CAV does not have any pathogenicity to older birds and plays only a role in young animals without maternal antibodies. Therefore vaccination in layers was thought to be not necessary. On the other side the immunosuppressive effects of field viruses are well known (ADAIR, 2000; JEURISSEN et. al., 1992).

   A special property of the CA virus seems to be the reason for the immmunsosuppression. It is expressing a protein that induces the so called suicide program (apoptose) in cytotoxic lymphocytes to protect the infected cells from the immune system. The lymphocytes die. The host by that means looses at least parts of it's control, which has been aquired by vaccination against Marek's disease. In addition cellular stress occurs and results in production of inflammatory mediators. The influence of these immunomodulatory molecules may lead to reactivation of MDV which means that the disease (Marek's) occurs more frequent than before. According to current knowledge the productive infection by both viruses (MDV and CAV) can result in nearly total depletion of immune cells. The mechanism described above is still theoretical and needs to be demonstrated in detail. The coincidence of Marek's disease in young layers with a simultaneous productive CAV infection makes the interaction of both virus systems probable. For vaccine strains of MDV and CAV so far no immunosuppressive effects were observed.

   In this context the problematic nature of the indirect infection of flocks by exposure to CAV-contaminated litter, which is a common practise, becomes clear. It can be assumed that the acute CAV infection does not only influence the protection against MDV, it may also open a window of opportunity for other infections. The real cause of the disease stays mostly undetected since screening for CAV-antibodies or CAV virus is rare, especially in layers.

5. A further reason for increase Marek's mortality which is often not realised is the presence of **stress** (stress hormones). Many of the Herpesviruses are known to be reactivated from latency by acute stress situations. If this principle is also true for MDV, the possibility must be addressed. It is common knowledge that stress induction in chickens happens easily. Lack of food or drinking water, high temperatures and mycotoxins are only a few examples of stress induction.

6. Finally the **evolution to new MDV variants** must be discussed as a possible cause of disease as it has happened in the US. Whether these new variants have an increased virulence (USA) or have just acquired the potential to evade the immune response within the flock is a still debatable question. The development of such variants is favoured by certain selective factors in the environment. One of these factors certainly is vaccination, especially in case of subprotective dosage or vaccination failures. A field infection occurring before establishment of a protective immune response causes a strong replication of the field virus in vaccinates and by this means produces a substantial genetic potential for adaptation to the selective pressure caused by the vaccination.

   Genetic resistance is certainly a second factor of evolution especially when the resistance is determined by only one gene. For MDV, many genes are probably involved in resistance, but the mechanisms are not

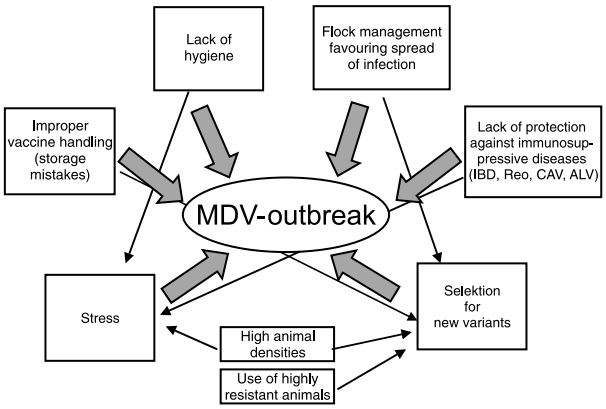
clear. If each gene functions by a different mechanism than each also produces it's own selective pressure on the virus.

The influence of all the factors mentioned, is increased by high animals densities which favour the rapid replication and by this the horizontal spreading of the virus. Mistakes in management do the rest to create a proper evolutional environment for the pathogen.

In summary: The situation in a given Marek's outbreak is usually not simple. A retrospective analysis by outsiders is normally not possible, since many factors must be taken into account to recognise the reasons and to take appropriate measures.

The coherence of all these factor is given schematically in Figure 2. Some of the factors do not only support the Marek's outbreak but also promote the selection of new variants. This can worsen the disease situation in addition.

Figure 2: Factors influencing outbreaks of Marek's field infections - an interactive network



Strategy and tactics

What to do, to control a possible Marek's infection optimally? The word control is used here intentionally, since avoidance is not yet possible.

Resulting from the points described above there is a number of rules which should be regarded as a matter of course but they are often not followed:

- Of highest priority should be proper vaccination. Before that one should ensure that the vaccine has been transported and stored at the appropriate temperature. This is especially important in case of cell associated vaccines. If the storage container does not contain enough liquid nitrogen the vaccine should not be used any more. During thawing and vaccination the recommendations of the vaccine producer should be followed strictly. The amount of vaccine prepared for use, should be not more than can be consumed within two hours. A mixture of products of different producers may cause damage to each of the vaccines and finally result in low efficacy. Inadmissible dilutions of the vaccine may also cause loss of protection. Diluent should be controlled accordingly.
- A single vaccination at the first day of life normally is sufficient for protection of the animals. WITTER could show, that a single vaccination which contains at least the RISPENS component, induces a sufficient immune response to protect against highly virulent strains.
- Many veterinarians believe in double vaccinations with an interval of 1 to 7 days. However, a scientific proof for the higher efficacy of this strategy is still missing. It may be assumed that the improvement of protection is just caused by the effect, that the second vaccination statistically ensures that more birds get vaccinated properly. The improvement of efficacy by increasing the virus titre per dose could not be shown as well. Nevertheless often high titres are used.
- Before housing new young chickens the building should be cleaned and disinfected as well as possible. The less amount of dust is present from the last passage of chickens the less risk of infection is present. In addition the tenacity of the virus is lower at high humidity.
- Contact between chickens of different age and of different breed should be avoided, especially when the immune status of these animals is different. The separation of buildings should be enough to make aerogen infections by vans blowing spent air out of an infected flock unlikely. Hatchery and rearing house should be kept strictly separated in all cases.
- The vaccination schedule should take into account all possible immunosuppressive diseases. Beside vaccinations against IBD and Reoviruses special attention should be given to CAV vaccination. The classical CAV-infection of a flock by litter management should be avoided. A proper prophylaxis against bacterial infections (Mycoplasma, Salmonella) may be of use too, since these infections may cause stress induced immunosuppression.
- Stress especially in presence of high MDV infection pressure should be avoided as far as possible. High animal densities, bad air conditioning, low hygiene (bacteria and mycotoxins) and many other factors are sources for stress development.
- Animals with typical clinical symptoms of Marek's disease should be removed rapidly from the flock and disposed of safely. Animals having a transient paralysis are known to replicate the virus in high amounts and are therefore a primary source of contact infection. Removal of these animals can drastically reduce infection pressure.

Current research and future perspectives

The current research aimed at fighting Marek's disease, is based on two pillars: research on resistance breeding and vaccine development. The genetic research localised a substantial number of loci in the chicken genome which are linked to MDV resistance. By now the system is complicated and confusing. Therefore substantial efforts in breeding are necessary to test all sensible combinations. The characteristics linked to these genetic loci are often still unknown. Here I like to refer to the term discussed at the beginning, genetic monoculture. Genes linked to MDV resistance are often coding for proteins of immunological relevance. A low genetic reservoir for these gene within the population puts a high selective pressure on the virus thus inducing the evolution of genetic variants. The effects of resistance breeding on susceptibility for other pathogens are still not exhaustively investigated.

For development of new vaccines meanwhile the era of molecular biology has begun. Genetic engineering methods will hopefully allow us to develop new vaccines

of high potency. According to current opinion it is senseless to develop new vaccine strains by attenuation of field isolates. It was shown, that these do not induce better or additional protection.

Prerequisites for the development of new vaccines is a good understanding of virus host interactions, which is still missing however. Despite extensive research for decades there is nearly no information concerning the following points:

- What is the route of entrance into the animal and what cells are infected primarily?
- In what cells does the primary viremia occur?
- In what cells does the virus go latent and how is latency established?
- How do tumors develop and what viral genes are responsible for oncogenesis?
- How does the immune system control a field infection after successful vaccination and protect from development of tumors?
- How does the virus enter the feather follicle of an infected animal and why is the virus cell free in this tissue (free infectious virus)?

These and many other questions can only be addressed by using the methods of molecular biology. Recently the DNA sequences of vaccine strains of each MDV serotype were published. This pool of information is a fundamental prerequisite for the understanding of infection mechanisms. The answers to the questions above will help to find new approaches to the development of vaccines against MDV.

The new vaccines themselves will also be influenced more and more by molecular biology. In cooperation with scientists from 6 countries, LAH is currently involved in an EU-project on development of next generation Marek's vaccines mainly based on methods of molecular biology. This group was recently successful in amplifying the whole genome of an attenuated passage of a highly virulent American isolate within bacteria. The naked DNA of these bacteria can be used to "infect" cells in vitro. This pioneering development will enable the rapid alteration of vaccine virus in future. It may help to optimise classical vaccine strains. Perhaps it may be possible to vaccinate chickens by using just naked DNA. Application of the latter method would solve all stability problems of the current vaccines.

The door to a new generation of MDV vaccines is now open but it is still not clear if it is possible to produce a vaccine which protects from infection and not only from disease.

Until new, more potent and user friendly vaccines will be developed, the well tested system of resistance breeding, vaccination, hygiene and good flock management must control the field situation as well as possible. The occurrence of vv^+ strains in the US makes clear, that even extreme situations in the field can be handled by optimised management.

Literature

For an overview about all aspects of the disease MAREK'S DISEASE, Supplement of World Poultry, 1997, is recommended.

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