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Marek's disease; current status in Ethiopia and its vaccination trial

TK Getahun*

Department of Agricultural Pharmacy, Animal Health National Research, Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia

*Corresponding author. E-mail: Temesgen.kassa@eiar.gov.et

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ABSTRACT

Marek's disease is a highly contagious, economically important paralytic viral disease of poultry and is seriously threatening the poultry industry of the world including Ethiopia. In the present study, Marek's disease virus isolated and characterized from clinically diseased chickens reared under different production systems in central Ethiopia. It is caused by herpes virus, belongs to the genus *Mardivirus*, family Herpesviridae. The virus causes massive destruction of lymphoid cells and macrophages inducing severe immunosuppression. Partial or complete paralysis is a fairly common symptom of Marek's diseases due to accumulation and proliferation of tumour cells in peripheral nerves. Marek's disease is diagnosed by isolation of the virus from tissue of infected chickens and viral antigen detection through available methods. Vaccination is the single most important method of controlling the diseases and preventing the development of tumours due to virus infection. Outbreaks still occur in vaccinated flocks under the right conditions due to a newly evolved highly virulent Marek's diseases virus strain. The Ripens vaccine appeared to perform marginally better than the RMIT vaccine. To determine the 50% protective dose of vaccine the challenge virus strain and dose, the genetic susceptibility and sex of the chickens and environmental factors should be considered.

Keywords: Marek's disease; Vaccine; Current status; Vaccination trial; Poultry; Herpes virus

INTRODUCTION

Marek's diseases exist in poultry producing countries throughout the world and probably every flock of chickens could become infected. The incidence of infection is surely much higher than the incidence of disease. Even in susceptible chickens, infection does not always induce clinical disease and in genetically resistant or vaccinated chickens infection may rarely cause overt disease. Marek's disease is a highly contagious lymphoproliferative disease in chickens. Birds like quail and turkeys can be affected naturally/artificially but chickens are more prone to this disease, as they are most important natural host for Marek's disease virus (Arulmozhi A et al. 2012).

Marek's disease is a cell associated, highly contagious, and economically important oncogenic or paralytic viral disease of poultry. It is caused by a herpes virus, which is distributed worldwide. The virus matures in the epithelium of feather follicles following infection and it sheds from these cells to the environment to infect other birds *via* inhalation. Marek's disease virus is oncogenic α-herpes virus that replicates in chicken lymphocytes and establishes a latent infection within $CD4^+$ T cells (Baaten BJ et al. 2004).

Marek's disease is one of the most common diseases of chickens which cause mononuclear infiltration to one or more of the peripheral nerves, gonads, iris, muscle, viscera, and skin (Bacon, et al. 2001). Marek's diseases commonly affect pullets between 12-24 weeks of age, but can infect broilers as early as 6 weeks of age. The incubation period ranges from 3-4 weeks to several months. Asymmetric progressive included paralysis of one or more of the extremities, Wing and limb drooping, and torticollis, vagal involvement will lead to dilatation of the crop and/or gasping and sometimes "grey eye" due to iris is involvement. Many birds die suddenly without symptoms. There are widespread nonspecific signs such as weight loss, paleness, anorexia, and diarrhea (Bacon LD et al. 2001).

Marek's disease is primarily controlled by live attenuated vaccines generated by repeated *in vitro* serial passage. Marek's disease vaccines as a class have been effectively protective against Marek's diseases and have provided greater than 90% protection in commercial settings (Baigent SJ et al. 2004).

Therefore, the objectives of this seminar paper are:

- To know the current status of MD incidence both within the worldwide and Ethiopia.
- To highlight methods in prevention and control of the disease.
- To know the most effective vaccine against Marek's diseases.

Current Status of Marek's Diseases and Its Vaccination Trial

Etiology: Marek's disease was first reported in 1907 by Josef Marek's and is characterized by T-cell lymphomas in the peripheral nerves or organs of 10-12 weeks old hens. Marek's diseases are caused by Herpes virus. Marek's disease virus belongs to the genus *Mardivirus*, a member of Alpha-herpesvirinae, subfamily, Herpesviridae (Biggs PM et al. 2012).

Three serotypes have been recognized such as serotype 1, which is more virulent, followed by serotype 2 and serotype 3, an avirulent Turkey Herpes virus. Serotype 1 (Marek's diseases virus-1) is more virulent and also comprises attenuated strains. Serotype 2 (Marek's diseases virus-2) is virulent virus isolated from chickens, while serotype 3 (Marek's diseases virus-3) is the Herpes Virus of Turkeys (HVT), which is used as a vaccine against Marek's diseases virus as a vaccine (Boodhoo N et al. 2016).

Among these three serotypes, serotype 1 Marek's diseases virus are oncogenic (tumour causing), and on the basis of their virulence, serotype 1 Marek's diseases virus can be classified further as mild (m), virulent (v), very virulent (vv), and very virulent plus (vv+) strains. Serotype 1 (Gallid herpes virus-2) is oncogenic, serotype 2 (Gallid herpes virus-3) and serotype 3 (Melegrid herpes virus-1) which is non-oncogenic in nature. The Marek's diseases virus serotypes are 50%-80% similar at DNA sequence level (Bumstead N et al. 2004).

Epidemiology

Prevalence of infection and disease: Marek's disease virus infection mainly occurs in domestic chickens and is ubiquitous among poultry populations throughout the world. The infection in other species is rare, but occasionally the disease occurs in turkeys and quails. In commercial chicken houses, where the infection is rampant, virtually all birds become infected, commonly within the first few weeks of life, although on occasions infection may be delayed. Because of the prevalence of serotype 1 viruses of varying pathogenicity and of non-pathogenic serotype 2 in the poultry house environment, birds can be infected with more than one MDV strain. Evidence suggests that the frequency of isolation of non-pathogenic viruses becomes higher as the age of the birds increases. Natural infection by non-pathogenic strains of MDV can provide immunity to subsequent infection by a virulent strain (Calnek BW, 2001).

Incidence of Marek's diseases increased from 1930's to 1950's, as poultry production increased and among flocks throughout the world. By 1960's Marek's diseases has caused heavy economic loss in poultry industry. Most chickens produce antibodies against Marek's diseases so they survive but virus is shed from skin and feather follicles. This dander remains infective for many months in dust. Congenital infection doesn't occur because chicks carry maternal antibodies for first week of a life (Calnek BW et al. 1979).

High mortality rate soon peaks up to 80% and later decline. Mortality rate could also vary from 1% to 50% in life span of chicken in population. Three factors such as:

- Virus's strain.
- Genetic composition of host.
- Age of host decides whether this infection leads to clinical disease.

Transmission of infection: The transmission of MDV occurs by direct or indirect contact, apparently by the airborne route. The epithelial cells in the keratinizing layer of the feather follicle replicate fully infectious virus, and serve as a source of contamination of the environment. The shedding of the infected material occurs approximately two to four weeks after infection, prior to the appearance of the clinical disease, and can continue throughout the life of the bird. The virus associated with feather debris and dander found in dust in the contaminated poultry house can remain infectious for several months. Although the inhalation of infected dust from poultry houses remains the most common route of disease spread, other less common mechanisms of indirect transmission, such as those involving darkling beetles (*Alphitobius diaperinus*), could also play minor roles in transmission. However, no evidence exists to suggest that vertical transmission of MDV occurs through the egg (Calnek BW et al. 1986).

Flock infection: Because of the ubiquitous nature of the infection and the ability to survive for long periods outside the host, flock infections usually occur early in the life of a bird. In addition, in most flocks, the hatched chicks usually have maternally derived antibodies. This antibody disappears in most chickens by three to four weeks of age. The rate of the spread of MD within a flock can vary greatly and depends on, among several factors, the level of initial exposure and the concentration of susceptible birds. A number of stress factors, including those from handling, change of housing and vaccination, are thought to increase the disease incidence. The existence of genetic resistance to MD among chickens has long been

recognized and the genetic constitution of the flock influences the outcome of MDV infection. The outcome of infection is also influenced by sex, as females are usually more susceptible to the development of tumors (Churchill A et al. 1969).

Pathogenesis

Pathogenesis of Marek's diseases has many unique distinguishing features. During the early cytolytic phase, lasting for approximately one week after infection, Marek's diseases virus causes massive destruction of lymphoid cells and macrophages inducing severe immunosuppression. After this period, Marek's diseases virus enters a lifelong latent phase in the $CD4^+$ and $CD8^+$ T cells where most of the viral gene expression is shut off. Some of the latently infected $CD4^+$ T cells are neoplastically transformed leading to the development of multiple lymphomas in visceral organs resulting in mortality from around 3-4 weeks post infection (Churchill AE et al. 1968). Paralysis, due to the lymphoid infiltration of the peripheral nerves, also occurs in some of these birds. In these different cell types, Marek's diseases virus remains cell associated and is spread by cell to cell transmission. However, from around 10 days after infection, the infection is transferred to the feather follicle epithelial cells, the unique cell type from where the cell free infectious virus is shed into the poultry house environment for long periods of time, acting as a source of infection to naive newly introduced birds. Biochemical and genetic studies have shown that *Meq* gene is main oncogenic factor of Marek's diseases virus (Duguma R et al. 2005).

Clinical Signs

Clinical signs of Marek's diseases include depression, crippling, weight loss, bursa/thymus atrophy, neurologic disorders, and rapid onset of T cell lymphomas that infiltrate lymphoid tissues, visceral organs, and peripheral nerves (Dunn JR et al. 2014). Paralysis occurs in wings and legs with neurolymphomatosis. Iris of one or both eyes in chickens becomes gray, because of ocular lymphomatosis. Cutaneous disease involves round, nodular lesions up to 1 cm diameter especially on feather follicles. Partial or complete paralysis is a fairly common symptom of Marek's diseases due to accumulation and proliferation of tumor cells in peripheral nerves (Figure 1) (Fadly AM et al. 1999).

Figure 1: Clinically diseased chickens suspected of Marek's diseases virus infection.

The chickens stretched their legs backwards and unable to walk.

MATERIALS AND METHODS

Diagnostic Methods

Virus isolation

Principle: There is a particular morphology of cells but when virus infects morphology of cell or tissue is changed, this is called as Cytopathic Effects (CPE).

Infection of Marek's diseases virus can be detected by the isolation of virus from tissue of infected chickens. Generally spleen cells, lymphoma cell suspension are used as a source. Monolayer cultures of chicken kidney cells or duck embryo fibroblasts/Chicken Embryo Fibroblast (CEF) are inoculated with cell suspensions. Generally 0.2 ml suspension is inoculated on duplicate monolayer grown in plastic cell culture dishes with control at 37°C in humid incubator containing 5% CO_2. The culture medium is replaced at 2 days interval; a cytopathic effect called as plaques, appears within 3-5 days and can be counted in 7-10 days (Fauquet CM et al. 1999).

Antigen detection/ELISA

Principle: Antibody and soluble antigen when interacts in aqueous solution forms a lattice that eventually develops into a visible line called precipitin. This process called precipitation method. In the method, antigen antibody interaction is viewed by the presence of chromogenic substrate, which is converted into a product by enzyme like Horseradish Peroxidase (HRP) tagged on antibody bound to epitope of Marek's diseases virus antigen. Marek's diseases virus antigen is detected in feather tips, which indicate the infection. Glass slide is prepared with 0.8% agarose containing Marek's diseases virus antiserum. Tips of small feathers are inserted vertically in agar and formation of radial zones of precipitation around feather tip indicates the presence of infection by Marek's diseases virus. ELISA gives the color intensity, on which basis; the presence of antigen can be detected including its severity in the sample (Fenner FJ et al. 1993).

Immunofluorescence

Principle: In this method, the antibody is tagged with fluorescent material like Green Fluorescent Protein (GFP). When GFP binds to target antigen, it gives fluorescence or a particular color at a specific wavelength, which shows the presence of antigen. It is visualized by fluorescence microscope.

Prevention and Control

Vaccination is the only known method to prevent the development of tumours when chickens are infected with the virus. However, administration of vaccines does not

prevent transmission of the virus, *i.e.*, the vaccine is not sterilizing. However, it does reduce the amount of virus shed in the dander, hence reduces horizontal spread of the disease. Marek's disease does not spread vertically. The vaccine can be administered to one day old chicks through subcutaneous inoculation or by in ova vaccination when the eggs are transferred from the incubator to the Hatcher (Frederick A et al. 1999).

Infection of the host and the transmission of the virus are not inhibited by the vaccine. This contrasts with most other vaccines, where infection of the host is prevented. A highly virulent strain would kill the host before the virus would have an opportunity to transmit to other potential hosts and replicate. Thus, less virulent strains are selected. These strains are virulent enough to induce symptoms but not enough to kill the host, allowing further transmission. Vaccination with live attenuated vaccines, introduced since the early 1970's, is the single most important method of control, although biosecurity measures and selection of birds for genetic resistance can also contribute towards the control of the disease (Gimeno IM et al. 2015).

Marek's diseases vaccines are very effective in protecting the birds against the disease, preventing clinical development of tumors, immunosuppression and paralysis and have played a major role in the sustainable growth of the poultry industry. However, the current Marek's diseases vaccines have a limited effect on viral infection and transmission. Hence, vaccinated birds continue to get infected and transmit the virus to the environment encouraging the evolution of Marek's diseases virus towards increased virulence.

Current Status of Marek's Disease in the World

The subclinical Marek's diseases investigated in current study with lymphomatic lesions in the livers and clinical form of Marek's diseases with involvement of sciatic nerves, spleens and livers showed an unexpected frequency of Marek's diseases in the broiler farms which is not desired due to its immunosuppressant effects in alive young birds and carcass condemnation of slaughtered broilers (up to 10%), meanwhile the vaccination failure and outbreaks of different infectious disease would be resulted.

Marek's disease is a great economic significance that was estimated to have mortality and condemnation losses of US$ 12 million and total losses of US$ 169 million in the United States (US$ 943 million worldwide) in 1984 when factoring vaccination and application costs and reduced production. Worldwide economic impact of MD at US$1-US$2 billion annually, though they acknowledged difficulty in verifying these costs (Gimeno, 2008).

- Marek's diseases outbreaks are occasionally reported in the literature. In general it is difficult to ascertain the global status of Marek's diseases the reasons for this difficulty:
- Marek's diseases is not a notifiable disease.
- Low-level losses after Marek's diseases vaccination are generally accepted and treated as normal, since it is known that vaccination failures occur at low frequency.
- Occurrence of Marek's diseases is often linked to financial claims between rearing companies and hatcheries or hatcheries and vaccine manufacturers, and often such cases are not made public. Since prevention of the disease requires optimal hygiene and management, besides several other measures, many Marek's diseases cases are not reported to avoid damaging the reputation of the company concern.

Factors that influence Marek's diseases incidence, such as coexistence of immune depressive agents and vaccination protocols.

In OIE's world animal health information database, some of the prevalence classifications for each country included:

- Disease present but without quantitative data.
- Disease present with quantitative data but with an unknown number of outbreaks.
- Disease suspected but not confirmed.
- Confirmed infection without clinical signs (Figure 2).

Figure 2: Disease distribution map for Marek's disease during the period July–December 2011.

RESULTS

In the United States, Marek's diseases incidence has continued to decrease during the last 10 years, reaching a record annual low in 2007 (0.0008%) as measured by Marek's diseases leukosis condemnation rates in young broilers at slaughter. Marek's diseases condemnations have been increasing during the last few years in several states, most notably North Carolina and Pennsylvania.

Virulence rank of MDV field isolates that have been path typed at ADOL over the years were plotted against condemnation rates based on the year of isolation. One can see the sharp increase in virulence of field strains in HVT vaccinated birds following introduction of both HVT and HVT-SB1 vaccines. This increase is somewhat more pronounced due to a change in the methods in which early path typing assays used maternal antibody-negative birds and maternal antibody positive birds.

It is interesting to note the large and sustained drop in MD condemnations between 2006 and 2007, which coincided with a large drop in condemnations in Delaware along with other nearby states. In Delaware alone, the number of young broilers condemned for leukosis dropped from 230,907 in 2006 to 18,015 in 2007. It is difficult to predict what may have led to this specific drop, but given the isolated geographic location it can likely be attributed to changes adopted by one or multiple broiler companies in the area. Possibly a change was introduced for vaccination procedures for a group of hatcheries. Another possibility could be changes that were related to increased disease surveillance and prevention in broiler breeders as part of ALV-J eradication efforts, following emergence of ALV-J within that area during the late 1990's (Figure 3).

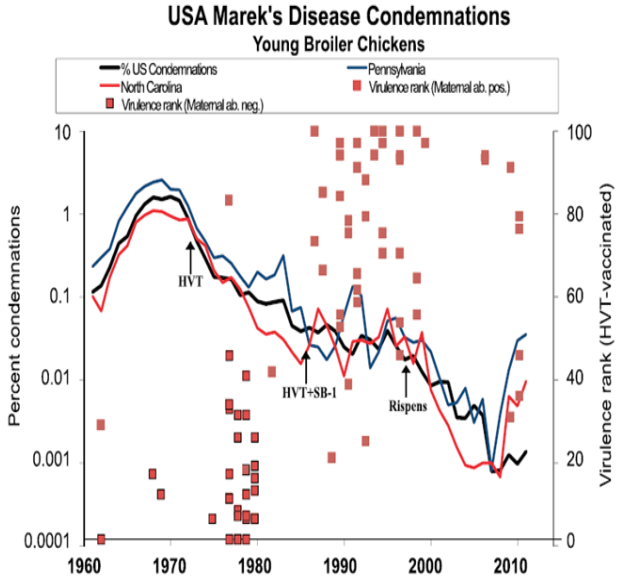

Figure 3: United States Marek's disease condemnations in young broiler chickens versus virulence rank of pathotyped field strains in HVT-vaccinated birds.

While MD has reached record low rates in the United States, as illustrated by leukosis condemnations in young broiler chickens at slaughter, frequent diagnosis of MD still occurs in many parts of the world, particularly French speaking Africa, Eastern Europe, East Asia, and Australia. Countries reported that MD incidence increased during the last 10 year. The most common reason perceived for this increase in broilers, layers, and breeders was due to coexistence of other immunosuppressive diseases. The next most likely cause for an increase was a mixture of miscellaneous ''other'' reasons, such as short rest period and downtime, poor cleanout, increased multi-aged farms, poor biosecurity, inappropriate nutrition, low PFU levels of vaccines, poor vaccine administration or handling, vaccine failure, missed vaccination, inappropriate vaccine chosen, due to higher virulent MDV (Figure 4).

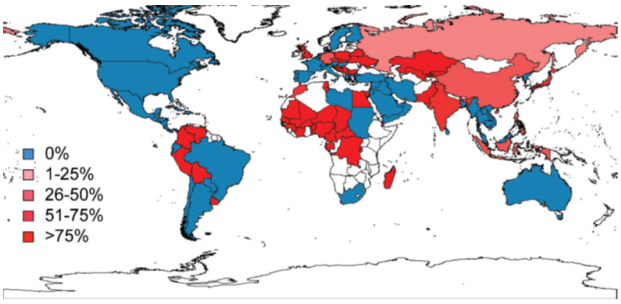

Figure 4: Countries reporting increased prevalence of MD during the last 10 years.

DISCUSSION

A decrease in MD incidence was reported from countries, most commonly reported for layer and breeder flocks. The most common reason suggested for the decrease in MD incidence among broiler and layer flocks, and second most common reason for breeder flocks, was the increased use of Ripens vaccination. Management related improvements were also highly suggested as reasons for decreasing MD incidence in all bird types, as well as miscellaneous ''other'' reasons, such as improved genetics, shorter growth period for broilers, better cleaning and disinfection, improved vaccine preparation and administration, doubling of vaccine dosage, more effective available vaccines or switching products, increased use of in ovo vaccination, higher PFUs of vaccines, introduced vaccination of broilers, and intensified education on correct vaccination protocols.

Current Status of Marek's Disease in Ethiopia

In Ethiopia MD was first diagnosed in 1983 and an incidence rate of 0.3% in industry poultry farm was reported for the year 1983-1986. Chickens infected with Marek's diseases virus carry the virus consistently for a long time, which increases the incidence and rate of virus-induced multi-organ tumors and increases its potential for horizontal transmission. (Zhang, et al. 2015).

Marek's disease is becoming a highly contagious and economically important oncogenic and/or paralytic viral disease of poultry and is seriously threatening the poultry industry of Ethiopia. It is becoming a growing concern that there is introduction of diseases of various etiologists into several poultry farms concurrent with importation of exotic breeds to the country. Among these threats, viral diseases like Marek's disease and infectious bursa disease are the major health constraints inflicting heavy losses.

An outbreak of Marek's disease in a commercial poultry farm in central Ethiopia mortality rate of 46%. The magnitude of morbidity and mortality on indigenous chickens in Ethiopia was nearly equal, indicating that Marek's diseases is highly fatal also to the local breeds.

The use of Marek's diseases vaccination using the available imported vaccines significantly increased the survival rate of the local breeds. In the present study, Marek's disease virus was isolated and characterized from clinically diseased chickens reared under different production systems in central Ethiopia. Identity of Marek's diseases virus was confirmed by using the recommended diagnostic techniques of cell culture, polymerase chain reaction, and sequencing.

Suspicion of Marek's diseases virus infections were previously reported in commercial poultry farms in Ethiopia where it has caused serious damage on poultry health and production. Marek's disease which is believed to have been introduced with the importation of exotic breeds of eggs and day old chicks is becoming a serious health problem of the poultry industry in Ethiopia. This represents a real threat to both local and exotic breeds in backyard and commercial farming systems. The present study confirmed that the circulating MD virus in Ethiopian chickens is the Gallid Herpes virus type 2 (Figure 5).

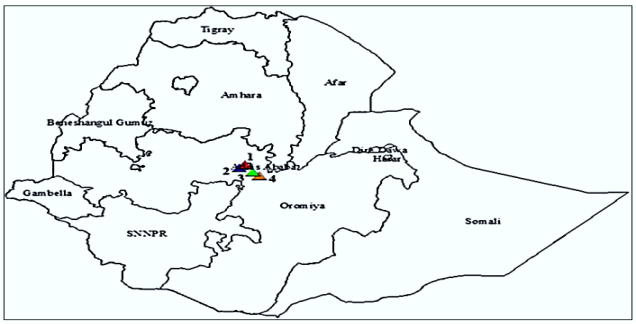

Figure 5: Spleen and feather samples have been collected during examination of MD suspected outbreaks from clinically diseased chickens.

Note: Red-Addis Ababa (1), Blue-Sebeta (2), Green-Debre zeit (3), and Orange-Mojo (4).

The economic loss incurred due to Marek's diseases is considerable particularly since modern poultry production in Ethiopia is at its infancy and such economic loss could discourage the farmer and ultimately seriously impair the promotion of poultry industry in the country. Marek's diseases is a major threat to poultry farming in Ethiopia at present chicken in Ethiopia are not vaccinated against Marek's diseases. Our observation indicates the MD should be considered as a diseases of economic significances in chicken production in Ethiopia and warrants more attention. New strain of MDV arises at different location throughout the world (Table 1).

Table 1: Mortality and associated economic loss due to Marek's diseases in a flock of 8500 chickens in Central Ethiopia.

Age (week)	No. of dead birds	Unit price (Birr)	Total loss (Birr)
8	162	14	2268
9	281	15	4215
10	302	16	4832
11	269	17	4573
12	312	18	5616
13	420	19	7980
14	465	20	9300
15	411	21	8631
16	340	22	7480
17	248	23	5704
18	211	24	5064

19	158	25	3950
20	167	26	4342
21	39	27	1053
22	42	27	1134
Total	3913		76142.00a

Note: [a]Approximately 9200 US$ (1 US$ = 8027 Birr) Source: Iobago and woldemeskel, 2004.

Vaccination of Marek's Diseases

Current vaccines used to control Marek's diseases include naturally avirulent MeHV-1 (turkey herpes virus, naturally avirulent GaHV-3 (serotype 2 MDV), and attenuated GaHV-2 (e.g., CVI988/Rispens).

Despite the large success of Marek's diseases vaccines, outbreaks still occur in vaccinated flocks under the right conditions. The default explanation for outbreaks of Marek's diseases in many cases is due to a newly evolved highly virulent Marek's diseases virus strain. History has shown that Marek's diseases virus does have an evolutionary trend toward greater virulence, which has required introduction of subsequent generations of Marek's diseases vaccines to maintain control.

However, other factors also play important roles in outbreaks and overall incidence of Marek's diseases. Reducing the vaccine titer can have detrimental effects whether the dosage is intentionally cut to save money or unintentionally reduced. Reducing the titer by using a partial vaccine dose is relatively common among broiler chickens in some parts of the world, which creates the potential for more rapid emergence of evolved strains.

Marek's disease is prevented by vaccination at 1 day or in-ovo. Both inactivated and tumour associated antigen can induce resistance to virulent Marek's diseases virus It is reported that non-oncogenic viruses (HVT and SB-1) are suitable as Marek's diseases vaccine, as these do not induce cytolytic infection in lymphoid organs.

Currently available commercial vaccines protect chickens against Marek's diseases; it still remains a serious threat due to increasingly frequent outbreaks of higher virulent strains of Marek's diseases virus combined with the incomplete immunity that is elicited by vaccination alone. Cell associated HVT has been more widely used because it is less expensive to produce and is more protective against Marek's diseases in chickens that are maternal antibody positive.

Mechanism of Vaccinal Immunity

Marek's diseases vaccine viruses establish a persistent infection which reduces early viraemia, after subsequent exposure to pathogenic strains, and protects against tumor formation and hence mortality so infection has no economic consequences. However, importantly, Marek's diseases vaccines do not prevent super infection by challenge viruses. Multiplication of the virulent challenge virus and its shedding from feather tissues still occurs. This has two major consequences. Firstly, virulent virus shed by vaccinated birds is still oncogenic to non-vaccinated birds. Secondly, the continued evolution of field viruses towards pathotypes of greater virulence (Figure 6).

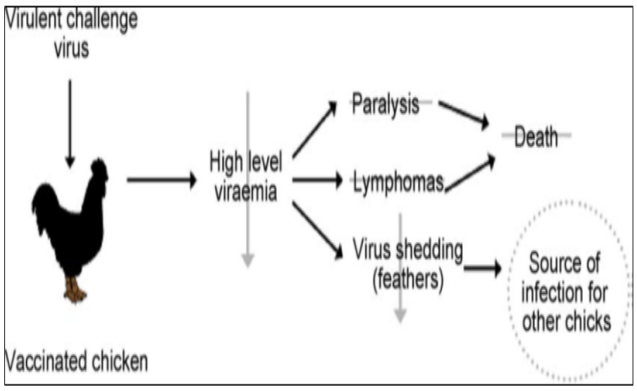

Figure 6: Protective effect of Marek's disease vaccination against virulent strains.

The black wording and arrows indicate the effects of infection by a virulent challenge strain of Marek's diseases virus in a non-vaccinated chick. The grey wording and arrows show the protective effect of vaccination.

Vaccine Storage and Administration

To use, cell associated vaccines must be thawed, prepared and administered according to strict guidelines to ensure minimum loss of titre. Inappropriate handling of vaccine can result in chickens not receiving the full dose, thereby decreasing the efficacy of protection. Vaccine virus is administered to day old chickens *via* the subcutaneous (neck) or intramuscular (leg) route using a semi-automated device. Many hatcheries now use an in ovo delivery system (Inovoject1, Embrex) to administer the vaccine to embrocated eggs 3 days before hatch (Figure 7).

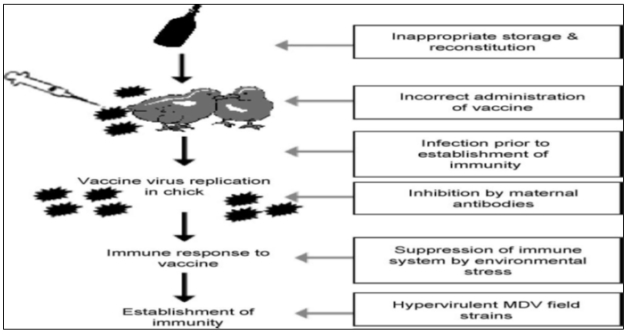

Figure 7: Causes of Marek's diseases vaccine breaks.

The black arrows summaries the induction of immunity against Marek's diseases, in chickens in which vaccination is successful. Vaccines breaks can be caused at various stages (grey arrows) from vaccine reconstitution, to establishment of vaccinal immunity. There are multiple causative factors (shown in the black boxes). Details are given in the main text.

Vaccination Trial on Marke's Disease

In the development of the RMIT serotype 1 vaccine against Marek's disease, a series of chicken trials have been conducted. Preparations of clone 60/2 at several different passage numbers were then assessed for safety trial (pathogencity), determination of its 50% Protective Dose (PD_{50}) and its efficacy compared with commercially available vaccines.

Safety Test

Chickens had been vaccinated with different dose with 2,000 PFU and 40,000 PFU. No gross lesions were observed throughout the trial but (5%) of vaccinated birds exhibited signs of dermatitis. Birds exhibiting dermatitis showed bursa and thymus atrophy, but the remaining vaccinated chickens were healthy and showed no gross signs of immune organ depletion. This was confirmed for bursa depletion when the bursa: Body weight ratios were examined and no significant differences between the vaccinated groups and the negative control. Thymus scores for both vaccine doses were slightly lower than the negative control. These results indicate that although there was no sign of serious immune organ depletion.

Marek's diseases lesion caused by vaccine strains of Marek's diseases virus or HVT has been described by several authors. The original Rispens (CVI-988) strain generally considered to be safe and of low pathogencity cause paralysis and neuritis in 88% of the highly Marek's diseases-susceptible strain of Rhode Island Red (RIR) chickens.

The pathogenicity of the CVI-988 strain for RIR chickens with classical symptoms of Marek's diseases in 28.5% of birds when inoculated with a high dose (6,640-12,000 PFU). Paralysis and endoneural inflammation in RIR chickens tested using the US strain of HVT, FC126. Another serotype 1 vaccine, the Md11/75C/R2 strain, caused lower body and bursa weights and resulted in up to 28% gross lesions.

Despite these findings, many of these vaccines are in common use throughout the world. The pathogenicity which is observed in highly MD susceptible lines, such as the RIR and the CSIRO SPF chickens used in this experiment, is not evident when used in commercial breeds of chicken which are usually less MD susceptible and May possess some protective maternal antibody against early MDV challenges.

Determination of 50% Protective Dose

The 50% Protective Dose (PD_{50}) is defined as the particular concentration of vaccine virus that induces protection in 50% of vaccinates. It is used to set an effective vaccinating dose and vaccine manufacturers will set different standards anywhere from <10-100xPD_{50}. There are many variables in the determination of the PD_{50} and these include the challenge virus strain and dose, the genetic susceptibility and sex of the chickens and environmental factors.

The PD_{50} determinations for a given vaccine varied depending upon the challenge virus, however the ranking for various vaccines would also change depending upon the challenge Virus used. For example, with the very virulent Marek's diseases virus Tun challenge strain, the Rispens (CVI-988) clone C derivative at passage 65 (CVI-988, CEF65 clone C) gave a PD_{50} of 5.2 and the HVT FC126 vaccine 60.8, however with a very virulent Marek's diseases virus Md5 challenge, PD_{50}'s of 19.9 and 7.6 Respectively were obtained. Demonstrating the complex nature of PD_{50} determinations and the difficulty in obtaining meaningful comparisons between vaccines, even when variables such as the challenge strain are constant.

Comparison of the RMIT Vaccine with Commercial Vaccines

The highest rate of protection (97.6%) was obtained for the Rispens vaccine when used alone, which was significantly greater than the RMIT vaccine when used alone (81.0%). However, protection induced by either vaccine when used in combination was not significantly different from each other or from a Maravac+TMC HVT combination. By contrast the Maravac and TMC HVT, when used in combination, provided significantly better protection than the Maravac+Steggles HVT combination. These results suggest that vaccine combinations which include the TMC HVT provide superior protection to that of the Steggles HVT vaccine. The relatively poor performance of the Steggles HVT vaccine may have been due to its significantly lower titre compared with TMC HVT (910 PFU compared with 8,000 PFU).

Unlike other vaccine groups, the two vaccine groups which received the RMIT vaccine (RMIT alone and RMIT

+TMC HVT) were not significantly different from the directly inoculated challenge group, suggesting that the RMIT vaccine in different does not protect birds from the immunodepressive effects of the Marek's diseases challenge as effectively as the other vaccines, or may have contributed to the immunodepression caused by the challenge virus.

The RMIT vaccine may provide superior protection under Australian conditions as it has been derived from a recent very virulent Australian strain of Marek's diseases virus, unlike the Ripens strain that was derived from a strain isolated in The Netherlands over 20 years ago before the advent of field strains of increasing virulence.

CONCLUSION

Marek's disease in commercial exotic chicken breeds in Ethiopia has revealed high morbidity and mortality (46%) in non-vaccinated birds. Marek's diseases incidence has continued to decrease during the last ten years In the United States. Frequent diagnosis of Marek's diseases still occurs in many parts of the world, particularly French speaking Africa, Eastern Europe, East Asia, and Australia. Marek's disease is a highly contagious lymphoproliferative disease in chickens. Caused by a herpes virus, which is distributed worldwide. High mortality rate soon peaks up to 80%. The virus matures in the epithelium of feather follicles and it sheds from these cells to the environment and birds become infect *via* inhalation. Vaccination with live attenuated vaccines is the single most important method of control to prevent the development of tumors when chickens are infected with the virus. Marek's diseases vaccines are very effective in protecting the birds against the disease. But there is vaccination failure due to virus evolutionary trend change toward a greater virulence, use of vaccine under dose, inappropriate rout of vaccine administration, inappropriate storage of vaccine.

Birds with dermatitis exhibited bursa and thymus atrophy whereas vaccinated birds without any signs of dermatitis (both high and regular doses of the RMIT vaccine) were healthy and showed no overt signs of immune organ depletion. The bursa: Body weight ratios were only moderately lower than the negative control birds and the thymus scores were approximately the same as that of the negative controls. This suggests that the few birds which acquired dermatitis may have developed immune organ depletion and were more susceptible to skin infection. However, the majority of birds did not show significant signs of immunodepression and did not develop dermatitis. No tumors were detected. Under field conditions other factors, such as the genetic characteristics of the chicken and maternal antibody status, circulating field strains and the environment, may play an important role in vaccine efficacy.

RECOMMENDATIONS

Based on the above conclusion, the following recommendations are forwarded:

- In depth studies should be done on investigation of the epidemiology of Marek's diseases.
- Knowledge on the use of vaccines against this disease should be exploited.
- To control and prevent the diseases using appropriate dose, rout of vaccine.
- Use vaccine combinations which provide superior protection against Marek's diseases.
- Infected chickens with virus should be isolate from the flock and good hygienic practices of poultry industry house are necessary.

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