Investigation Study of Turkey Meningoencephalitis (TME) Vaccine Failure: Causes and Solutions in the Field

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Abstract

During 2008 and as in previous years, severe outbreaks of turkey Meningoencephalitis (TME) occurred in many turkey farms in Israel. Many of the outbreaks occurred in turkey flocks in spite of vaccination, suggesting vaccination failure. Serological examination of vaccinated flocks revealed in many cases very low hemagglutination inhibition titers against the TME virus. To provide answers to the questions raised, a long term investigation study was carried out under laboratory and field conditions. In this report we describe the investigation of the problem of vaccination failure from the epidemiological aspects to the solution of the problem at field level. Based on the investigation and trial results, it was concluded that the vaccination failure of TME vaccines was probably related to technical problems and not to the vaccine itself. Some of the recommendations for vaccination included: The use of only high quality TME vaccines; pre-cool the diluent; use the vaccine within 60 minutes after dilution; vaccination is best carried out during the day; adapt the size of the needle to the size of the bird to be injected.

Key words: Turkey, Meningoencephalitis, TME, Epidemiology Vaccination Failure, Turkey Vaccination.

INTRODUCTION

Turkey Meningoencephalitis (TME) is an infectious viral disease that causes disease only in turkeys under natural conditions. The causal agent of TMV is an arbovirus which was isolated by Komarov in 1960 (1) and classified by Portfield in 1961 to the flaviviridae family (2). Up to now the TME virus (TMEV) has been isolated and reported only in Israel and South Africa (3). TMEV is transmitted by three different blood sucking mosquitoes, *Aedes* spp., *Culex* spp., and *Culicoides* spp. (4, 5).

In contrast to other Flaviviridae-Arboviruses, TMEV under natural conditions affects only turkeys and does not affect humans or other mammals as in the case of West Nile Virus or other viruses from this group such as the Ntaya, Bagaza, Ilheus or Tembusu viruses (6, 7, 8). TMEV causes a serious disease in turkeys characterized by neurological signs including reluctance to walk, paralysis, weakness of the neck, incoordination and mortality. Outbreaks of TME are more common during autumm, spring and summer but in some regions the disease may appear throughout the year. TME is usually observed in turkeys between 8-20 weeks of age but in hot areas, outbreaks of TME have been observed as early as 5 weeks of age. Morbidity can be high and mortality rates may reach 30% of the flock or even higher.

The disease is prevented mainly by the use of a live-attenuated vaccine developed by Prof. Iankonesku in 1975 (9, 10). Since the introduction of the TME live-attenuated vaccine, a constant decline in the number of cases of TME has been observed (Figure 1). Despite the extensive use of the vaccine and the gradual decrease in the number of outbreaks, about

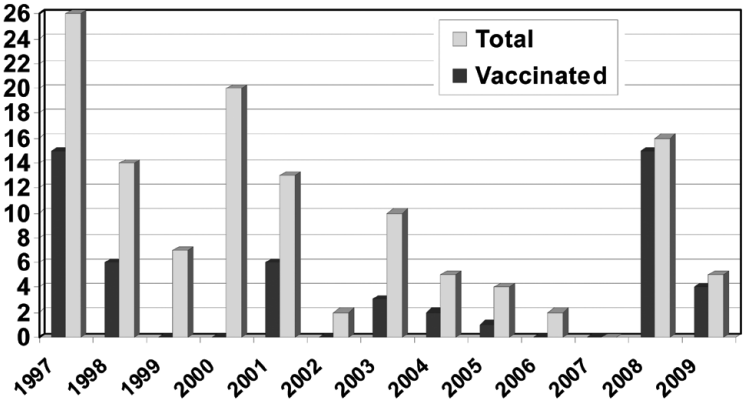

Figure 1. Number of TME outbreaks in vaccinated and non vaccinated turkey flocks in Israel. (Years 1997-2009) Data provided by the Regional Poultry Laboratory-North.

50% of the oubreaks have ocurred in vaccinated flocks. In 2004 a preliminary study was carried out by the Department of Avian diseases at the Kimron Veterinary Institute in order to investigate changes in the genome and the antigenic characteristics of TMEV (11).

In 2008 a severe outbreak of TME occurred affecting more than 16 turkey farms where 94% of the affected flocks were reported to have been vaccinated against TME with the live vaccine. This mass vaccination failure raised some critical questions as to whether the vaccination failure was related to: emergence of new strains of TMEV; specific batch or different batches of TME vaccine, or were due to technical vaccination problems.

To provide answers to the questions raised, a long term investigation study was carried out under laboratory and field conditions. In this report we describe the investigation of the problem from the epidemiological aspects to the solution of the problem at field level.

MATERIALS AND METHODS

Turkey Meningoencephalitis Vaccine: The vaccine used in all turkey farms in Israel is based on the basic original seed prepared by Prof. Ianconescu in 1976 at the Kimron Veterinary Institute. Isolate No. 931-(71)-KVI was passed 4 times in Japanese quails by intracerebral injection. After the last passage the virus was passaged 11 times in Japanese quails kidney cell tissue culture and one more passage in chicken embryos, in this obtaining way the basic vaccinal virus (JQ4K11E1) for the preparation of the commercial vaccines in israel.

The TME vaccine is produced in Israel by two companies and both use the same original basic seed (JQ4K11E1). The titer used in the vaccine is $x10^3$ EID/50 per dose. The vaccine must be diluted in a special diluent containing a special alkaline buffer before use.

The turkeys involved in the TME cases came from different hatcheries and different breeding farms. Affected turkeys included Nicholas and hybrid lines.

Analysis and description of the problem at the field level.

Relevant information on TME outbreaks was collected from affected farms and analyzed. The data concerning the TME vaccine included: Date of purchase, batch numbers, storage conditions of the vaccine and date of use, the application of the TME vaccine, as well as details concerning the composition of the vaccinating teams.

The health status of the flock prior to vaccination was documented as well as the serological status of the flocks before and after TME vaccination. Information regarding the line of turkeys, the age of the flock at vaccination and the age of the flock at the time of the TME outbreak was recorded. Morbidity and mortality data from each of the affected flocks was collected.

Evaluation of the protection provided by the vaccine.

A study was carried out in order to obtain accurate data about the ability of the vaccine to provide protection against new TME isolates. One of the new TME isolates (2004) (11) and the original virulent TME (1965) virus were injected intracerebrally in two different groups of newborn mice (7, 10). All mice showing neurological signs post infection were humanely sacrificed and their brains harvested to prepare a 1:10 suspension in PBS. The suspension prepared for each of the TME isolates was used for a challenge experiment according to the following protocol: Sixty turkey poults of 8 weeks of age were separated in four groups of 15 birds each. The turkeys were then vaccinated with commercial vaccine and then challenged with the original or new isolates of TME:

Group	Vaccinated with TME vaccine	Challenge TMEV
1	Non-vaccinated	Original TME isolate
2	Non-vaccinated	New TME isolate
3	Vaccinated	Original TME isolate
4	Vaccinated	New TME isolate

All turkeys in groups 3 and 4,were vaccinated at the same time with the same batch of vaccine by the same vaccination team. During the trial, all the birds were maintained in isolation. Three weeks post vaccination and before challenge all birds were bled and their sera tested for antibodies to TME by the hemagglutination inhibiton (HI) test as described previously (12). Challenge in turkey poults was carried out by intra-cerebral injection with 0.2 ml of the infected mice brain suspensions.

Serological survey of healthy turkey flocks vaccinated with different batches of TME vaccine by different vaccination teams.

To test the serologic response of vaccinated turkey flocks, blood samples from 25 turkeys were taken from the wing vein from each flock tested. The blood samples were taken at least 3-4 weeks post vaccination and sent to the Poultry Health Regional Laboratories to be tested for antibodies by the HI test.

Effect of the injection site on the immune response and duration of titers after TME vaccination.

It has been recommended that the vaccine be injected by intramuscular (IM) injection into the leg muscle (9,10). However under field conditions the TME vaccine is often injected into the breast muscle. To assess the immune response on the vaccination site, a control trial was carried out comparing injection into leg muscle versus breast muscle. Sixty turkey poults of 6 weeks of age raised in isolation were used: Twenty five blood samples were collected before vaccination to determine the serological status for TME. The turkey poults were then divided into 4 groups of 15 turkey poults each as follows:

Group 1: Vaccinated with TME vaccine by IM route into the leg muscle.

Group 2: Vaccinated with TME vaccine by IM route in the breast muscle.

Group 3: Vaccinated with TME vaccine twice (49 and 70 days) by IM route in the breast muscle.

Group 4: Maintained as the non-vaccinated control group.

Turkeys from groups 1, 2 and 3 were vaccinated with 0.5ml vaccine dose.

All turkeys were bled and tested for antibody levels using the HI test by the Poultry Health Laboratory after 70, 90 and 112 days post vaccination.

Evaluation of different methods of application of the TME vaccine by vaccination teams under field conditions.

To test and evaluate the application of the TME vaccine under field conditions three trials were carried out on different farms.

Field trial 1: Two methods of vaccination by the same vaccination team using the Abic TME vaccine (TME live vaccine Batch No. 209089) (Abic, Israel) were compared using 100 turkeys from two buildings at the same farm.

In the first method each turkey was vaccinated by slowly injecting a full dose of vaccine intramuscularly. After vaccination these turkeys were separated off by a fence from the rest of the flock. The second method was that used routinely, where injector teams vaccinate turkeys randomly by catching birds from one side of the buiding to the end of the building.

Three weeks after vaccination, 25 blood samples were taken from each group of turkeys and tested y the HI method for antibody response.

Field trial 2: The comparison of the serologic response after vaccination with TEM vaccine by two different vaccination teams using the same vaccine was evaluated.

In order to evaluate the quality of application of the same vaccine batch by two different professional vaccination teams, two houses containing about 2000 turkeys each were used. One hundred turkeys were vaccinated by the controlled method and separated by a fence from the rest of the flock. The rest of the turkeys in each house was vaccinated by two different vaccination teams using the routine method as described in the previous field trial. Three weeks after vaccination 25 blood samples were taken from the 100 birds vaccinated under controlled conditions and 25 blood samples from tukeys vaccinated by each one of the two teams respectively from two different houses. The blood was sent to the Regional Poultry Health Laboratory for assesment of the serological response by the HI method.

Field trial 3: The effect of different vaccination procedures and techniques on the serologic response of turkeys was test-

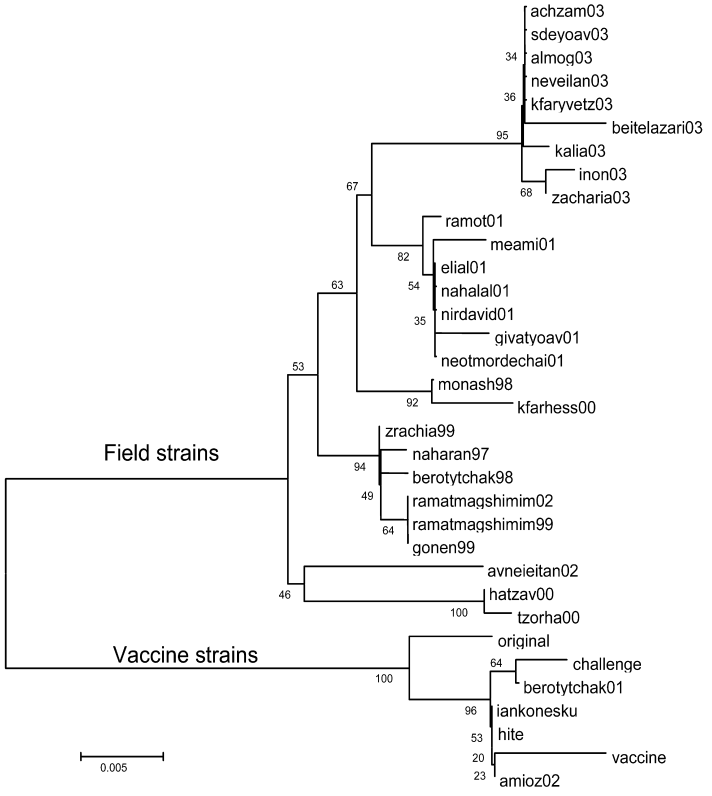

Figure 2. Phylogenetic tree of TMEV's isolated from 1997 to 2004. (Study carried out by the Department of Avian Diseses at the Kimron Veterinary Institute)

ed. Six houses of 1000 turkeys each from the same farm were injected with the same batch of vaccine by the same vaccination team.

The different parameters tested were:

1. Day versus night vaccination: Ten vaccinators were employed catching and injecting the birds working from one side to the other side of the building. This technique enables a very fast vaccination of the turkeys and is most commonly used at night because turkeys do not move in the dark, enabling the vaccinators to approach and inject the birds as they are sitting on the litter floor.
2. Vaccination by the common method of 10 vaccinators catching and injecting the birds compared to vaccination by 6 presentors and 3 injectors.
3. Use of short needle 1/4" compared to long needles 3/8" for injection.
4. Stability of the vaccine after dilution: 30 minutes after dilution in cold diluent versus 90 minutes after dilution in cold diluent.
5. Use of one dose of vaccine using a double volume of diluent (1ml versus 0.5 ml).

RESULTS

Collection of background data.

The data provided by the Northern-Regional Poultry Health Laboratory showed that there had been a decrease in the number of outbreaks of TME from 1997 to 2007. However in 2008, TME was diagnosed in 16 turkey farms with 15 out of the 16 outbreaks reported in vaccinated flocks (Figure 1). Research carried out by the Deparment of Avian Diseases at the Kimron Veterinary Institute demonstrated changes in the Gene E (responsible for the envelop proteins) of the TMEV (Figure 2) (11).

Challenge trial with the original and new TME isolate in vaccinated turkeys.

The results of the challenge test after vaccination comparing the original TMEV from 1964 with a new isolate from 2004 showed that the vaccine provided a 100% protection against challenge with the original TMEV and 93% protection against the new TMEV isolate. The mortality in unvaccinated turkeys was 100% for the new 2004 isolate as compared to 46% mortality for the 1964 TME strain (Table 1).

Serologic survey

The serological survey showed that in vaccinated turkey flocks, the percentage of turkeys with HI antibody titers of 1:40 or lower, ranged from 10 % to 90% (Figure. 3), (HI titers of 1:80 or above are considered as protective).

Table 1. Summary of the challenge trial and protection provided by the classic vaccine against original and new isolates of TME

Groups	HI Titers before challenge	Mortality post Challenge (%)
Non-Vaccinated Challenged 1964 TME isolate	5.7	7/15 (46%)
Non-Vaccinated Challenged 2004 TME isolate	3.1	15/15 (100%)
Vaccinated Challenged 1964 TME isolate	581	0/13 (0 %)
Vaccinated Challenged 2004 TME isolate	667	1/13 (7.6%)

Trial carried out by Abic's technical team and facilities

Breast versus leg vaccination trial

No significant differences in the antibody titers were obtained after vaccination by the IM route in the leg muscle compared to the breast muscle (Figure. 4). In vaccinated turkeys with a high antibody titers, a second vaccination with TME vaccine, did not induce higher antibody titers or improved the duration of immunity tested up to 112 days post vaccination.

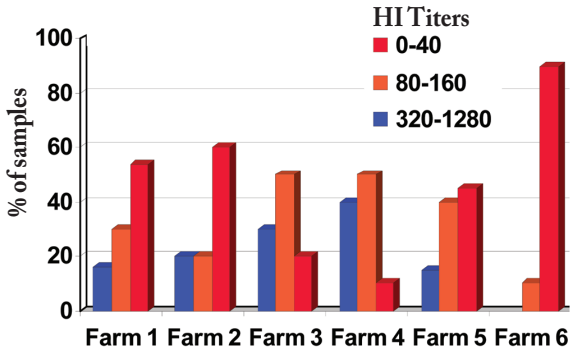

Figure 3. Results of the serologic survey carried out in commercial turkey flocks after vaccination with TME vaccines applied by different vaccination teams.

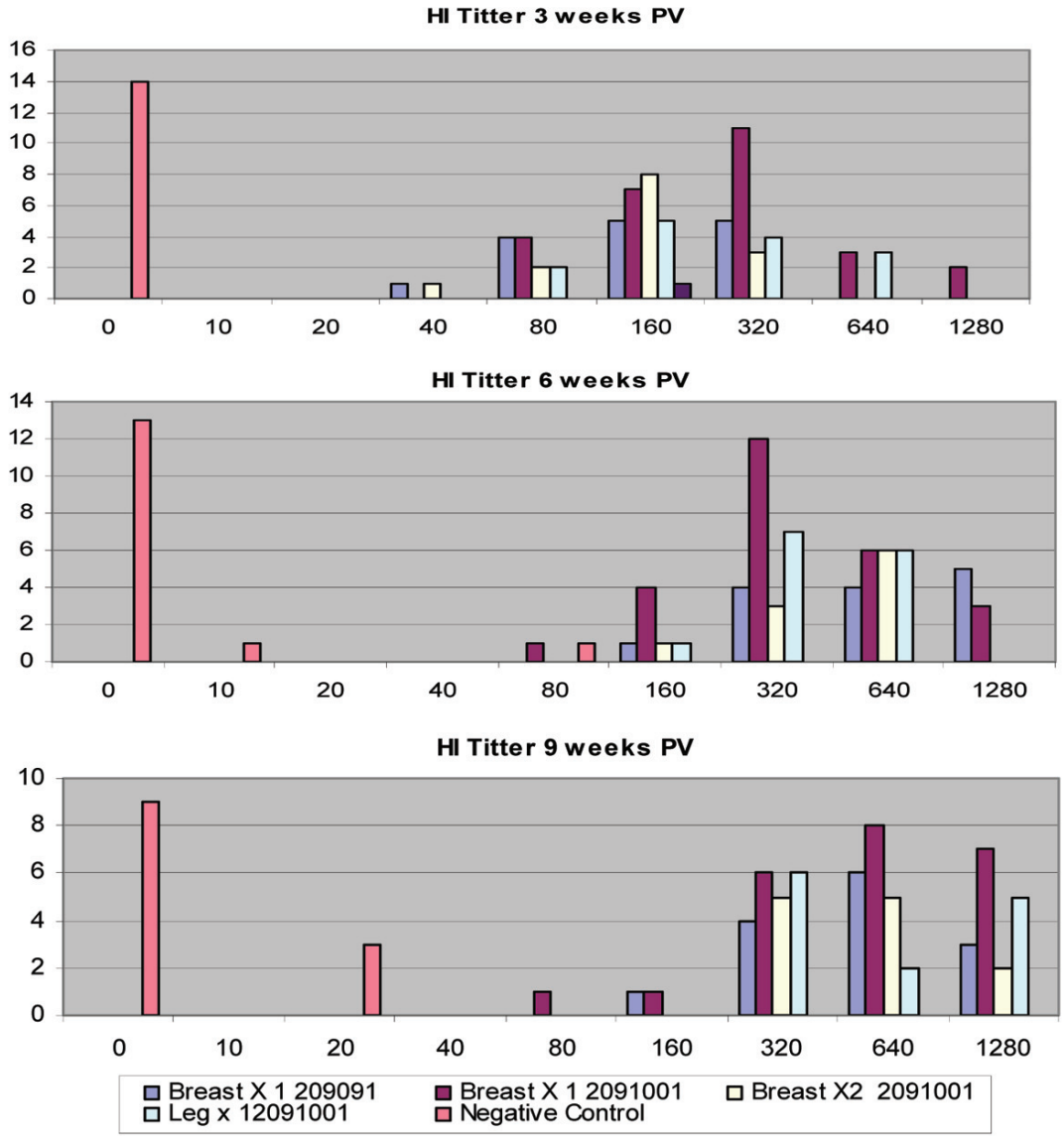

Figure 4. Effect of the vaccination site (Leg vs. Breast) on the immune response to TME vaccine Antibody titers were measured by the HI test after 3, 6 and 9 weeks post vaccination

Trials to assess the effect of different methods of vaccination on the serologic response of turkeys under field conditions:

The results of field trials 1 and 2 indicate that only 4% of the turkeys vaccinated under controlled conditions had HI antibody titers below the protective titer of 1:80. Among the turkeys vaccinated using the routine vaccination technique (fast vaccination at night), 16% to 20% of the samples showed HI antibody titers 1:40 or below (Figure 5).

Trial three was carried out to identify the key technical factors involved in the vaccination failure of the TME vaccine. Results are summarized in figure 6. The immune response after vaccination of the turkeys with TME vaccine was highly dependent on the catching and vaccinating procedures, the "classical" approach based on catching and vaccinating by the same person was compared to a new approach based on 6 presenters and 3 vaccinators.

TME vaccination at night by 10 vaccinators resulted in 70% of the turkeys having HI titers equal to or less than 1:40. In contrast, TME vaccination carried out during the day by 10 vaccinators resulted in 21% of the turkeys with HI titers equal to or less than 1:40.

Turkeys vaccinated during the day by 6 presenters and 3 vaccinators using a 1/4" (short) needle, developed the following pattern of antibody titers. 42% of the turkeys had HI titers of 1:80, and 58% of the turkeys had titers between 1:160 to 1:320, no birds were found with HI titers lower than 1:80.

Preparation of TME vaccine 90 min before application and vaccination during the day by 6 presenters and 3 vaccinators resulted in 42% of the turkeys having titers of 1:80 and 58% with titers between 1:160 to 1:320 results were similar as those observed in the group of turkeys vaccinated with the TME vaccine injected within 30 minutes from preparation. In the group of turkeys vaccinated with the same TME vaccine during the day by 6 presenters and 3 vaccinators using a 3/8" (long) needle resulted in 8% of the turkeys having HI titers of 1:80 and 92% of the turkeys having HI titers between 1:160 to 1:640.

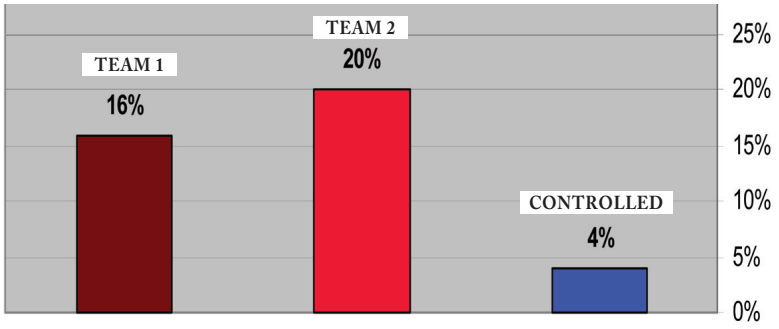

Figure 5. Controlled application of TME vaccine compared to rapid (routine) vaccination by two different teams. Columns in figure represent the % of samples with antibody titers below the minimum protective level (HI titer of 1:80).

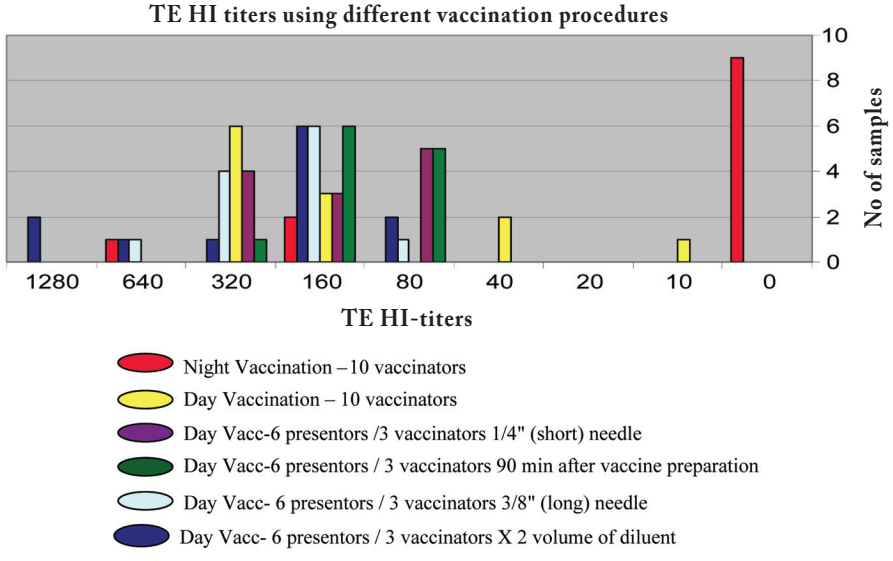

Figure 6. Correlation between number of samples and HI titers to TME after vaccination using different vaccination techniques and approach.

TME vaccination during the day by 6 presenters and 3 vaccinators using a double dose of diluent (1ml) resulted in 16% of the turkeys having HI titers of 1:80 and 84% of the turkeys with titers between 1:160 to 1:1280.

DISCUSSION

Vaccination of turkeys is aimed at preventing the economic damage caused by diseases caused by different pathogenic agents. Success of vaccination in commercial poultry farming depends on several critical factors such as the quality of the vaccine, antigenic characteristics, storage and transportation conditions, the vaccination programs and the method and quality of application of the vaccines (13). Most of the vaccine companies, produce, store and market their vaccines under controlled conditions of quality control and good manufacturing processes (GMP).

Good quality vaccines are able to provide a good immune response and protection only when applied properly using the correct vaccination program. However, even the best vaccine will not be able to provide a high and uniform immune response and protection of the vaccinated flocks when the quality of application is poor.

In order to induce a good immune response, it is critical that the attenuated vaccine reach the target organs, proliferate and stimulate the immune system to generate the specific antibodies required for protection (13, 14). Most of vaccination failures reported involved a small number of cases or were usually related to a specific batch of vaccine, or a local error in the preparation or application of the vaccine.

Many different technical factors may be involved in vaccination failures including vaccination at an early age, presence of high levels of maternal antibodies, use of an inappropriate vaccination program, inadequate dose of the vaccine, antigenic differences between the vaccine used and the field pathogenic agent, detrimental storage conditions or incorrect preparation or application of the vaccine (15).

Under commercial conditions, serological screening usually reveals a wide range of titers within the flock or among different flocks. When the quality of a vaccine is affected by erroneous production process, all or most of the vaccinated birds will show a low or lack of response to the vaccine. When the vaccination titers are not checked after vaccination the lack of immune response will not be observed, thus resulting in a mass vaccination failure among the birds.

Low and irregular titers of antibodies after vaccination with the TME vaccine have been reported for many years. Speculation has been raised with regard to the factors involved. The wide range of immune response within and between vaccinated flocks suggested that the vaccination failures may be related to some technical problem however this hypothesis until now has not been verified. Of the vaccination failure reported in 2008, 94% of the affected flocks were vaccinated with the attenuated TME vaccine using different batches and different manufacturers.

This investigation considered many possible reasons for the TME vaccination failure: The filogenetic differences of new TME isolates as reported by C. Noah and S. Perek (11) raised the possibility that the antigenic variations in the new isolates of TME could be related to the mass vaccination failure. The study carried out to provide an answer to this question, showed that the classic vaccine when applied properly, was able to provide an adequate level of protection against an intra-cerebral challenge with the new TME (2004) isolate despite the filogenetic distance between the vaccine virus produced in 1965 and the new TMEV isolates. An interesting finding of this trial was the increased virulence of the new 2004 TME isolate compared with the original 1965 TMEV suggesting that higher antibody titers may be required to provide better protection against the new and more virulent isolate of TME.

The serological screening of healthy vaccinated turkey flocks, showed a very wide range in the HI antibody titers within and among flocks indicating that some turkeys responded well to the vaccine while other birds in the same house or flock did not respond at all. The number of turkeys with very low levels of antibodies to TME (< 1:80) ranged from 10% of the turkeys in one farm to 90% in another farm suggesting a great variation in the quality of application of the TME vaccine. The experimental trials including controlled vaccination of 100 birds followed by "fast" vaccination as routinely done by different vaccination teams using the same batch of vaccine, clearly demonstrated that the vaccination failure was related to one or more critical technical errors by the vaccination teams and was not due to the vaccine quality or its immunogenicity.

Controlled vaccination of 100 birds using a bird by bird slow application approach, proved to be the key for a successful vaccination with TME vaccine, but did not fully explain what were the crucial issues involved in the failure during the routine vaccination procedure. The differences in the antibody response obtained between "night" and "day" vaccinations were informative, demonstrating that vaccination during

the night is probably the most important factor involved in the vaccination failure. It appears that during the night many birds remain unvaccinated. Even vaccinating in daylight with 10 vaccinators catching and injecting the birds (fast vaccination), about 20% of the turkeys remained unvaccinated. Vaccinating with short needles of 1/4" length was poorer than that obtained using 3/8" long needles. These results are probably due to the fact that part of the vaccine may have spilled out after the injection process leaving some birds unvaccinated or with lower antibody titers.

It has been reported lately that the circadian rhythm may affect the immune response to Hepatitis B vaccination (16), Further studies should be considered to evaluate the effect of vaccination during night on the immune response to other vaccines such as TME vaccine.

One of the goals of this study was to test the viability of the vaccine following a relatively long time (90 minutes) after dilution of the freeze-dried vaccine. The results of our study showed that use of the vaccine 90 minutes after dilution were poorer than those obtained in the flocks vaccinated within 30 minutes after dilution of the vaccine, suggesting a slight reduction in the viability of the vaccine. Based on the results obtained, we conclude and recommend to use only pre-cooled diluent and to use the vaccine within 60 minutes after preparation.

An important finding of this study was that dividing the vaccination teams in presenters and vaccinators at a ratio of 2:1 respectively is by far the best method of vaccination under field conditions.

Based on the investigation and trial results, it can be concluded that the vaccination failure of TME vaccines was probably related to technical problems and not to the vaccine itself. In order to obtain the best possible results using TME live vaccine under field conditions the following points should be taken into consideration:

1. Only high quality TME vaccines should be used.
2. Pre-cool (7°C) the diluent.
3. Use the vaccine within 60 minutes.
4. Vaccinate during the day when it is possible to follow and monitor the vaccinating team quality of work.
5. The vaccination approach based on 2 catchers and presenters for every vaccinator is the preferred method.
6. Adapt the size of the needle to the size of the bird to be injected.

During the last decade chicken and turkey farms have grown from relatively small farms to large farms with many birds, making the vaccination of the flocks a very technical and complicated issue that must be clearly understood in order to prevent vaccination failures. This study is an example of the close relationship between the quality of application of the vaccine and the success or failure of vaccination in poultry.

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