*Original Article*

# Protection Against *Mycoplasma gallisepticum* in Layers Immunized with Recombinant Fowl Poxvirus Vaccine Followed by Live F Strain Vaccine

**Somsak Pakpinyo[1*] Arithat Limsatanun[1] Kreangkrai Sangthongdang[2]**

**Marcelo Paniago[3] Roberto Soares[3]**

***Abstract***

Generally, one administration of either a recombinant fowl poxvirus-*Mycoplasma gallisepticum* (rFPV-MG) or a live F strain (FMG) vaccine is recommended in layer flocks. This study aimed to assess MG protection when the rFPV-MG and FMG vaccines were given to layers as opposed to a single vaccination with FMG vaccine. One hundred 8-week-old layer chickens were divided into 4 groups as follows: group 1 received FMG at 11 weeks of age, group 2 received rFPV-MG at 8 weeks of age and FMG at 11 weeks of age, groups 3 and 4 served as positive and negative controls, respectively. At 14 weeks of age, groups 1-3 were challenged with a Thai MG strain. The protection was monitored by evaluating body weight gain, thoracic air sac and tracheal lesions and MG detection. Sera of 8- to 18-week-old layer chickens were collected and tested for MG-antibody response. During 12-16 weeks of age, five birds in each group were necropsied to evaluate gross thoracic air sac lesion and histopathologic tracheal lesion. At 16 and 17 weeks of age, MG were re-isolated and identified by using RAPD PCR assay to differentiate strains. Results revealed that during 14-18 weeks of age, the birds receiving rFPV-MG and FMG vaccines had a superior body weight gain and prevented colonization of the MG challenge strain. In conclusion, the birds receiving rFPV-MG and FMG vaccines provided appropriate performance compared with the other MG-challenged groups. The combination of rFPV-MG and FMG vaccination program could be used for MG protection in MG endemic area.

**Keywords:** layer chickens, live FMG vaccine, *Mycoplasma gallisepticum*, protection, rFPV-MG vaccine

[1]*Avian Health Research Unit, Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*

[2]*CEVA Animal Health (Thailand) LTD., Bangkok 10400, Thailand*

[3]*CEVA Animal Health Asia Pacific Sdn. Bhd. Selangor, Malaysia*

***Correspondence**: somsak.pa@chula.ac.th*

## Introduction

*Mycoplasma gallisepticum* (MG) infection is known as a chronic respiratory disease (CRD) (Ley, 2008). MG disease can be transmitted by horizontal and/or vertical routes. MG-infected chickens show respiratory signs including rales, sneezing, conjunctivitis, nasal and ocular discharges and even death (Pakpinyo and Sasipreeyajan, 2007). Moreover, adverse effects on feed conversion rate, weight gain and egg production have been observed (Ley, 2008). Airsacculitis caused by MG infection results in an increase in condemnations at the processing plants. Various diagnostic methods such as serum plate agglutination, ELISA, microbial culture and polymerase chain reaction (PCR) have been used to identify the infection (Kleven, 1998; Lauerman, 1998). Prevention and control are important procedures for the management of MG infection. Vaccination is one of the procedures that can control and reduce economic loss from MG infection, significantly without depopulating flocks (Ferguson-Noel et al., 2012). There are several types of vaccine including live, killed and a recombinant fowl poxvirus (rFPV)-MG vaccine (Ley, 2008). Commercial live vaccines are 6/85, ts-11 and F strains (Ferguson-Noel et al., 2012). The FMG strain, of which several data showed protection against MG challenge, persists for a long time in the upper respiratory tract in chickens, is able to displace infection from the MG challenge strain and can prevent egg production losses in MG infected flocks (Glisson and Kleven, 1984; Glisson and Kleven, 1985; Cummings and Kleven, 1986; Kleven, 1998; Branton et al., 2002; Evans et al., 2012). However, the FMG vaccine is more virulent compared with other strains and possibly egg transmitted in birds receiving this strain during egg production period (Lin and Kleven, 1982; Whithear, 1996). Presently, there are a few publications that determined the rFP-MG vaccine in chickens (Zhang et al., 2010; Leigh and Branton, 2013). The rFPV-MG vaccine induces the expression of proteins that protect against MG challenge. The advantages are simultaneous immune response against fowl poxvirus, no shedding of MG microorganisms and no detectable circulatory antibody. These features allow the application of the DIVA concept as vaccinated birds showing antibody response have to be infected with MG (Kleven, 1998). However, one study described that the rFP-MG did not provide any protection of the respiratory and reproductive systems against virulent MG R strain (Ferguson-Noel et al., 2012). Interestingly, giving vaccination with rFPV-MG and re-vaccination with FMG to pullets at 45 weeks old showed good results including no adverse effects on egg production and egg shell quality (Leigh and Branton, 2013).

From the advantages of FMG and rFPV-MG vaccines, this study proposed an alternative prevention program of a combination of rFPV-MG vaccine and live FMG vaccine in layer chickens. The aim of the present study was to determine the proposed program in layer chickens compared with birds vaccinated with only live FMG vaccine in terms of safety, performance and protection against MG challenge.

## Materials and Methods

***Animals and Experimental Designs*:** One hundred, one-day-old commercial female layer chickens, Isa Brown breed, free of *Mycoplasma gallisepticum* and *M. synoviae* (MS) were provided by registered commercial layer breeders in Thailand. At 8 weeks of age, all pullets were equally and randomly divided into 4 groups as follows: group 1 received Cevac® MGF vaccine, group 2 received Vectormune® FP-MG and Cevac® MGF vaccine, groups 3 and 4 served as positive and negative controls, respectively. All pullets were raised in wired cages in isolated rooms and provided with feed and water *ad libitum*. At 8 weeks of age, the palatine fissures of twenty pullets of each group were swabbed to test by MG and MS polymerase chain reaction (PCR) and their wing veins were bled for MG and MS serology. At 11 weeks of age, 20 pullets of groups 2 and 4 were bled for MG serology. At 12 and 13 weeks of age, five pullets of each group were euthanized and necropsied to blindly evaluate gross air sac lesion and histopathological tracheal lesion scores. At 14 weeks of age, each bird of groups 1, 2 and 3 was challenged with a Thai isolate of MG (AHRU 54/46) that was 100 µl in volume with approximately 1 x $10^6$ colony forming unit (CFU) through nasal and ocular drop. Group 4 served as the sham negative control. Clinical signs including respiratory signs and mortality were observed for 4 weeks. Fifteen, ten and five pullets at 14, 16 and 18 weeks of age, respectively, were bled for MG serology. All fresh sera were collected and properly identified. At 16 and 17 weeks of age, seven and five pullets, respectively, were swabbed through the trachea for MGF strain RAPD PCR re-isolation. At 15, 16 and 18 weeks of age, five pullets of each group were euthanized and necropsied to blindly evaluate as previously described. Body weight gain of all groups was recorded during 8-14 weeks of age (post-vaccination period) and 15-18 weeks of age (post-challenge period) (Table 1). All handling procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Chulalongkorn University, Thailand number 13310001.

***Vaccination procedures:*** At 8 weeks of age, the pullets of group 2 were vaccinated with one dose of Vectormune® FP-MG (CEVA Santé Animale, France) by wing web administration and at 5 d post-vaccination the vaccine taken was checked by assessing pox takes. At 11 weeks of age, all the pullets of groups 1 and 2 were vaccinated with one dose of Cevac® MGF (CEVA Santé Animale, France) by ocular drop.

***Media:*** Frey's broth medium (GIBCO Diagnostics, Madison, Wisconsin) was used in this study as previously described (Kleven, 1998). The sterile broth was supplemented with 15% swine serum, dextrose, cysteine, nicotinamide adenine dinucleotide, penicillin, thallium acetate, and phenol red and referred to as Frey's broth medium supplemented with swine serum (FMS).

***MG challenge organism:*** The MG challenge organism used in this study was isolated from the culling of broiler breeder hens in the central part of Thailand in 2003. The affected broiler breeder hens aged 50 weeks old showed respiratory signs including rales, sneezing, conjunctivitis and ocular discharge, and were submitted to Faculty of Veterinary Science, Chulalongkorn University, Thailand for diagnosis. Airsacculitis at the thoracic air sacs was observed, swabbed and placed in FMS broth. MG was isolated and identified by direct immunofluorescent assay (Kleven, 1998) using fluorescein-conjugated rabbit antiserum provided by S.H. Kleven (Department of Avian Medicine, University of Georgia, Athens, GA) and MG PCR assay. The MG isolate, named as AHRU 54/46, was propagated, aliquoted and stored at -70°C to be used in MG challenge study. One frozen aliquot was determined for MG titration as colony forming unit (CFU) by serially ten-folded dilution procedure on FMS agar.

**Table 1** Experimental designs in this study

| Group | Experimental designs at each age (weeks of age) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **8** | **11** | **12** | **13** | **14** | **15** | **16** | **18** |
| 1 (FMG alone) | - | FMG | N | N | C | N | N | N |
| 2 (Combination program) | rFPV-MG | FMG | N | N | C | N | N | N |
| 3 (Positive control) | - | - | N | N | C | N | N | N |
| 4 (Negative control) | - | - | N | N | C | N | N | N |

C and N mean MG challenge and necropsy of 5 birds/group, respectively. Blood collection at 8, 11, 14, 16 and 18 weeks of age, tracheal swabs at 8, 16 and 17 weeks of age and body weight gain during 8-14 and 15-18 weeks of age were done.

***MG and MS Serology:*** Fresh sera were used to detect antibody against MG by serum plate agglutination (SPA) using Rapid Plate Agglutination (Soleil Sarl, France) following the manufacturer's instructions. Briefly, thirty µl of serum was mixed with thirty µl of antigen and then incubated at room temperature for 1-2 min before result was read. Negative and positive sera were determined in each test. Subsequently, the serum samples were frozen at -20°C to be further tested at once for MG antibody by ELISA.

ELISA: sera were tested with MG and MS commercial ELISA test kits (BioChek®, Netherlands) following the manufacturer's instructions. Briefly, diluted sera were added onto an MG antigen-coated plate, incubated, washed, and peroxidase labeled. Then, an anti-chicken antibody was added. After incubation, the plate was again washed before adding a substrate and then a stop solution was added. The plate was read in an ELISA reader manufactured by Labsystems Multiskan MS Type 352, Finland. Optical density of the negative and positive controls and the samples was calculated and interpreted according to the manufacturer's recommendations. For the interpretation, titer levels of 0-667 or greater than 668 were negative or positive reactors, respectively.

***Evaluation of lesion score:*** Air sac lesion score: the left and right thoracic air sac lesion scores were grossly evaluated as the following criteria described by Kleven et al. (1972): 0 = no air sac lesion was observed; 1 = lymphofollicular lesions or slight cloudiness of air sac membrane were found; 2 = air sac membrane was slightly thick and usually presented small accumulations of cheesy exudates; 3 = air sac membrane was obviously thick and meaty in consistency, with large accumulations of cheesy exudates in one air sac; and 4 = lesions were observed as same as 3, but 2 or more air sacs were found. An average of the left and right thoracic air sac lesion scores was recorded.

Tracheal lesion score: the trachea of each chicken was collected and divided into 4 parts; proximal, 2 middle and distal parts. Each part of the trachea was microscopically evaluated and the lesion score followed the criteria described by Yagihashi and Tajima (1986): 0 = no significant changes were observed; 1 = small aggregate of cells (mainly lymphocytes) was found; 2 = moderate thickening of wall due to cell infiltration and edema commonly accompanied with epithelial degeneration and exudation were present; and 3 = extensive thickening of wall due to cell infiltration with or without exudation was determined. An average of each part of tracheal lesion score was recorded.

***Preparation of MG DNA for PCR and randomly amplified polymorphic DNA (RAPD):*** MG DNA preparation was done using a previously described protocol (Fan et al., 1995) with adaptation. Briefly, 2 ml of FMS broth swabbed from the palatine fissure or trachea was incubated at 37°C for 2 hrs. After incubation, the cultured broth was equally divided into two 1.5-ml tubes; the first tube was determined for PCR assay and the remaining tube was stored at -70°C until used. The cultured broth was centrifuged to collect the pellet, washed twice with phosphate-buffered saline (PBS) and suspended with 25 µl of PBS. The pellet was then boiled for 10 min, placed on ice for 5 min and centrifuged. The supernatant containing MG DNA was collected and stored at 4°C for further PCR assay. For RAPD assay, only positive samples by PCR assay were further processed as follows: the cultured broth of the second tube stored at -70°C was thawed and 200 µl was transferred into 1.8 ml of sterile FMS broth, then incubated at 37°C until the MG cultured broth changed from pink to orange-yellow in color. All 2 ml of MG isolated broth culture was prepared and used as MG DNA as previously described.

***PCR assay:*** Amplified reaction was performed in a 25 µl volume using a modified described protocol

(Lauerman, 1998). Each PCR mixture consisted of 2.5 mM $MgCl_2$, 1 mM dNTP (Fermentas, USA), 10 pmole primer MG 13F (5′-GAGCTAATCTGTAAAGTTGGTC 3′), 10 pmole primer MG 14R (5′-GCTTCCTTGCGGTTAGCAAC 3′) for MG PCR or 10 pmole primer MSL-1 (5'-GAAGCAAAATAGTGATATCA-3') and 10 pmole primer MSL-2 (5'-GTCGTCTCCGAAGTTAACAA-3') for MS PCR (Qiagen, Germany), 1.25 U of Taq polymerase (Promega, USA), and 2.5 µl of MG DNA. Each reaction was performed concurrently with the S6 strain (ATCC 15302) as a positive control and with distilled water as a negative control. The amplification conditions were 94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min for 40 cycles. The final extension cycles were 72°C for 5 min. The amplification product was 185 base pairs (bps).

***RAPD assay:*** The primer set for RAPD analyses was modified from Ley et al. (1997). Briefly, Geary primer set (Geary et al., 1994) was performed in a 25 µl volume and each RAPD mixture consisted of 2 mM $MgCl_2$, 1 mM dNTP (Fermentas, USA), 500 ng primer 1254 (5′ CCGCAGCCAA 3′) (Qiagen, Germany), 2.5 U of Taq polymerase (Promega, USA) and 0.5 µl of MG DNA containing approximately 25 to 50 ng DNA. The amplification conditions were performed starting with four cycles of 94°C for 5 min, 36°C for 5 min, and 72°C for 5 min, ending with 30 cycles of 94°C for 1 min, 36°C for 1 min, and 72°C for 1 min and finally a cycle of 72°C for 10 min. Each reaction was performed concurrently with the F strain (Cevac® MGF vaccine) and Thai MG strain as a positive control. PCR banding pattern was analyzed by agarose gel electrophoresis.

***Gel electrophoresis:*** A volume of 10 µl of the amplified DNA products was loaded into 2% agarose (Pharmacia Biotech AB, Uppsala, Sweden) gels and separated by agarose gel electrophoresis. The gel was stained with 0.5 µg/ml ethidium bromide and analyzed by a gel documentation system (Vilber Lourmat, France).

***Statistical analysis:*** Evaluation of lesion scores was statistically analyzed using the nonparametric Kruskal-Wallis test and Mann Whitney U test. Data were analyzed by using SPSS Statistics 17.0. Statistical significance was determined at $p \leq 0.05$.

## *Results*

In the birds allocated to group 2 (rFPV-MG vaccine followed by FMG vaccine), at 5 d after rFPV-MG vaccination, pox takes were found in all vaccinated birds and the pox takes of all birds disappeared at 2 weeks post-vaccination. After the vaccination of FMG, vaccine reaction was not observed in any vaccinated birds in groups 1 (receiving FMG vaccine) and 2.

After challenge, respiratory signs including serous ocular discharge, sneezing and respiratory rales were observed in the birds of group 3 (positive control) and lasted for 2 to 3 weeks post-challenge.

Body weight gain of all groups is summarized in Table 2. Basically, from 8 to 14 weeks of age, the period before challenge, similar body weight gain was observed among the groups ranging from 14.06-14.48 Kg. However, after challenge, from 15 to 18 weeks of age, group 2, which received the combination of MGF and rFPV-MG vaccines, showed better body weight gain when compared with groups 1 and 3, whereas group 4 (negative control) had the highest body weight gain.

**Table 2** Body weight gain (Kg) of each group at different ages post-vaccination (8-14 weeks of age) and post-challenge (15-18 weeks of age)

| Group | Body weight gain (Kg) | |
|---|---|---|
| | 8-14 | 15-18 |
| 1 | 14.44 | 2.63 |
| 2 | 14.26 | 2.94 |
| 3 | 14.06 | 2.39 |
| 4 | 14.48 | 3.68 |

The serology against MG was determined by SPA and ELISA. At 8 weeks of age, all birds were negative by SPA and ELISA. Before challenge, at 11 weeks of age, all groups showed negative results by both SPA and ELISA. At 14 weeks of age, the results in both tests turned positive. Groups 3 and 4 remained negative as expected at 11 and 14 weeks of age. After challenge, groups 1, 2 and 3 showed positive results by SPA at 16 and 18 weeks of age. By ELISA, groups 1 and 3 were negative at 16 weeks but positive at 18 weeks of age. Group 2 was positive in both weeks. As expected, group 4 showed negative results against MG by SPA and ELISA at all ages (Table 3).

**Table 3** Serology results against MG determined by using SPA and ELISA at different ages (8-18 weeks of age)

| Group | SPA | | | | | ELISA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | 11 | 14 | 16 | 18 | 8 | 11 | 14 | 16 | 18 |
| 1 | 0/20* | 0/20 | 14/15 | 10/10 | 5/5 | 0/20* | 0/20 | 1/15 | 0/10 | 5/5 |
| 2 | 0/20 | 0/20 | 13/15 | 8/10 | 5/5 | 0/20 | 0/20 | 9/15 | 10/10 | 4/5 |
| 3 | 0/20 | 0/20 | 0/15 | 10/10 | 5/5 | 0/20 | 0/20 | 0/15 | 0/10 | 1/5 |
| 4 | 0/20 | 0/20 | 0/15 | 0/10 | 0/5 | 0/20 | 0/20 | 0/15 | 0/10 | 0/5 |

* Number of positive samples/total number of tested samples

Before challenge, the gross air sac lesion scores showed that no or very mild air sac lesions were observed at 12 and 13 weeks of age in the vaccinated groups. However, after challenge, at 18 weeks of age, group 3 (positive control) showed significantly higher lesion score as compared to the other two vaccinated groups (Table 4). The histopathological tracheal lesion scores carried out before challenge at 12 and 13 weeks of age were not statistically different among the groups. However, after challenge, at 18 weeks of age, similarly to the findings of the gross lesions, group 3 (positive control) showed significantly higher lesion score as compared to the other two vaccinated groups (Table 4).

The PCR results of 30 birds at 8 weeks of age (prior to vaccination) were negative for MG and *MS*. At 16 and 17 weeks of age, MG DNA could be re-isolated from groups 1, 2 and 3. None of MG DNA was detected in group 4, which served as the negative control group (Table 5).

At 16 weeks of age, two weeks after challenge, in group 1, vaccinated with only the FMG vaccine, 5 out of 7 birds were positive for the FMG strain and 2 were positive for the MG challenge isolate. In group 2, vaccinated with a combination of rFPV-MG and FMG vaccines, only the FMG strain was detected. Finally, in group 3, the positive control, only the MG challenge isolate was detected. At 17 weeks of age, in all samples from groups 1 and 2, the FMG strain was detected while in group 3 only the challenge MG isolate was found.

**Table 4** Gross air sac lesion scores and histopathological tracheal lesion scores at 12, 13, 15, 16 and 18 weeks of age (n=5)

| Group | Gross air sac lesion scores | | | | | Histopathological tracheal lesion scores | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | 13 | 15 | 16 | 18 | 12 | 13 | 15 | 16 | 18 |
| 1 | 0 | $0.10 \pm 0.22^{a}$ | $0^{a}$ | $0.10 \pm 0.35^{a}$ | $0.60 \pm 0.55^{a}$ | $1.25 \pm 0.18^{a,b}$ | $1.25 \pm 0^{a}$ | $1.30 \pm 0.33^{a,b}$ | $1.30 \pm 0.27^{a}$ | $1.50 \pm 0.47^{a,b}$ |
| 2 | 0 | $0^{a}$ | $0.90 \pm 0.65^{b}$ | $0.50 \pm 0.50^{a}$ | $0.20 \pm 0.45^{a}$ | $1.60 \pm 0.29^{a}$ | $1.40 \pm 0.22^{a}$ | $1.25 \pm 0.18^{b}$ | $1.85 \pm 0.22^{b,d}$ | $1.40 \pm 0.42^{b}$ |
| 3 | 0 | $0^{a}$ | $0.60 \pm 0.65^{a,b}$ | $1.10 \pm 0.74^{a}$ | $1.60 \pm 0.55^{b}$ | $1.25 \pm 0.18^{a,b}$ | $1.60 \pm 0.34^{a}$ | $1.60 \pm 0.14^{a}$ | $2.50 \pm 0.47^{c}$ | $2.20 \pm 0.21^{c}$ |
| 4 | 0 | $0^{a}$ | $0^{a}$ | $0.70 \pm 0.45^{a}$ | $0.70 \pm 0.45^{a}$ | $1.20 \pm 0.21^{b}$ | $1.25 \pm 0.18^{a}$ | $1.10 \pm 0.14^{b}$ | $1.60 \pm 0.29^{a,d}$ | $1.35 \pm 0.29^{b}$ |

$^{a,b}$ = Different superscripts in the same column means significant difference ($p<0.05$).

**Table 5** MG PCR at 8, 16 and 17 weeks of age and re-isolation of FMG strain at 16 and 17 weeks of age by RAPD PCR

| Group | MG PCR | | | FMG strain | |
|---|---|---|---|---|---|
| | 8 | 16 | 17 | 16 | 17 |
| 1 | 0/6* | 7/7** | 5/5 | 5/7† | 5/5 |
| 2 | 0/6 | 7/7 | 5/5 | 7/7 | 5/5 |
| 3 | 0/6 | 7/7 | 5/5 | 0/7 | 0/5 |
| 4 | 0/6 | 0/4 | 0/5 | ND†† | ND |

* Number of MG positive samples/total tested samples
** Only MG positive samples were further differentiated for FMG.
† Number of FMG strain positive samples/total PCR tested samples
†† ND = not determined

## *Discussion*

The MG prevention and control can be done by using vaccines in combination with appropriate biosecurity procedures. The advantages of rFPV-MG and FMG vaccines have been shown in several publications (Glisson and Kleven, 1984; Branton and Deaton, 1985; Cummings and Kleven, 1986; Zhang et al., 2010; Evans et al., 2012; Ferguson-Noel et al., 2012; Leigh and Branton, 2013). However, this is the first publication to evaluate the efficacy of combined vaccination program with rFPV-MG followed by FMG.

This study measured the body weight gain before and after challenge in order to evaluate the safety of the vaccines and their efficacy. Before challenge, the body weight gain of the vaccinated and non-vaccinated groups were quite similar, suggesting that no deleterious effect on growth was induced by the vaccines. In fact, as the birds vaccinated only with the MGF vaccine (group 1) showed slightly higher body weight gain compared to the birds receiving rFPV-MG and FMG vaccines (group 2) and the positive control group (group 3), the absence of differences observed between groups 1 and 2 did not allow the conclusion that the use of a rFPV-MG vaccine prior to MGF vaccine could reduce the vaccine reaction.

However, after challenge, groups 1 and 3 showed lower body weight gain as compared to groups 2 and 4 (negative control). This result suggests that the group vaccinated with the combination of rFPV-MG and FMG vaccines had better protection against the negative impacts of the MG infection as compared to the group receiving only the MGF vaccine.

Seroconversion of the birds 3 weeks after the vaccination with rFPV-MG vaccine was not detected by SPA and ELISA. This result is in agreement with previously published articles (Zhang et al., 2010; Leigh and Branton, 2013). However, the pox takes observed in all vaccinated birds at 5 days post-vaccination demonstrate that there was replication of the recombinant vaccine, hence expression of the protective proteins. As a consequence, these proteins induced the cell-mediated immunity (Zhang et al.,

2014). In addition, no vaccine reaction was observed compared with the other groups. This result is in agreement with the study of Zhang et al. (2010).

Interestingly, although the number of positive samples tested by the SPA assay was not different, at 14 weeks of age, the birds immunized with rFPV-MG followed by FMG vaccine showed much more positive antibody samples and higher titer samples (data not shown) measured by MG ELISA as compared to the birds immunized with FMG alone. This result suggests a prime-boost effect of both MG vaccines. In fact, it is necessary to consider that the circulatory antibodies do not correlate with the MG protection, suggesting that the local antibody plays an important role in the protection against the negative effects of MG infection (Abd-El-Motelib and Kleven, 1993; Avakian and Ley, 1993; Gaunson et al., 2006).

Generally, the commercial ELISA test kit and SPA mostly detect immunoglobulin IgG and IgM, respectively. IgM against MG is the first immunoglobulin produced in early immunization or infection (Kempf et al., 1994) and IgG is produced in later period, which are in accord with the results of SPA assay and ELISA of the positive control group in this study.

In the present study, the air sac and tracheal lesion scores were evaluated after the FMG vaccination and MG challenge. The results of the gross and microscopic lesions assessed before challenge showed that there was no difference between the groups receiving the FMG vaccine, associated or not to rFPV-MG vaccine, and the non-vaccinated groups. Although these results could suggest the fairly good safety of this vaccine strain, these results are different from those observed by Pakpinyo et al. (2013) in which birds vaccinated with the F strain had significantly higher histopathological lesions as compared to non-vaccinated birds. Despite being more aggressive compared with other live MG vaccines, several data showed the good protection against MG challenge of the F strain (Cummings and Kleven, 1986; Whithear, 1996; Levisohn and Kleven, 2000; Evans et al., 2012; Ferguson-Noel et al., 2012). Similar results were observed in the present study. FMG, with or without the rFPV-MG vaccine, protected the air sac and trachea lesion when compared with the MG challenge group. Unfortunately, group 4 (negative control) had tracheal lesion scores without respiratory clinical signs during 12-18 weeks of age, possibly caused by the inappropriate ventilation of experimental room.

When comparing the protection induced by the FMG vaccine alone or in combination with the rFPV-MG vaccine, taking the macro- and microscopic lesions as the criteria, the results are not as clear as it was suggested by the results of body weight gain after challenge. Actually, taking the gross lesions in the air sacs into consideration, although there was slightly higher lesion score at 15 weeks of age (1 week post-challenge), there were no significant differences between these two groups at 16 and 18 weeks of age. Likewise, the microscopic lesions in the trachea were slightly higher at 16 weeks of age (2 weeks post-challenge), but no significant differences at 15 and 18 weeks of age were found.

When assessing the protection by the detection and differentiation of MG strains in the respiratory tract, the combination of MG vaccines showed better performance as compared to the FMG vaccine alone. In fact, at 16 weeks of age, two weeks after challenge, 2 out 7 birds vaccinated with only the FMG vaccine were positive for the MG challenge isolate (71% of protection). Alternatively, all birds vaccinated with the combination of rFPV-MG and FMG vaccines were positive for the F strain (100% of protection). At 17 weeks of age, only the vaccine strain was detected in both groups.

Although these results are in agreement with Levisohn and Dykstra (1987), who showed that the FMG vaccine did not protect the colonization against the MG challenge strain, they differed from the results obtained by Pakpinyo et al. (2013), who showed complete protection after vaccination with the F strain vaccine. These results suggest that using both types of vaccines possibly improves the protection in layer flocks especially in MG endemic area.

This is the first article describing the vaccination program of rFPV-MG vaccine followed by FMG vaccine. The results showed that this program could increase the body weight gain of layer chickens and protect them against MG challenge strain. Nevertheless, the cost of vaccines should be considered. Furthermore, the MG control and prevention in layer flocks require an appropriate biosecurity combination with a proper vaccination program.

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# บทคัดย่อ

## การป้องกันโรค *มัยโคพลาสมา กัลลิเซพติกุม* ในไก่ไข่ที่ได้รับการกระตุ้นด้วยวัคซีนรีคอมบิแนนท์ไวรัสฝีดาษไก่ตามด้วยวัคซีนเชื้อเป็น *มัยโคพลาสมา กัลลิเซพติกุม* สายพันธุ์เอฟ

*สมศักดิ์ ภัคภิญโญ[1*] อริธัชย์ ลิ้มศตนันท์[1] เกรียงไกร แสงทองแดง[2] มาร์ซีโล พาเนียโก[3] โรเบอร์โต ซอแรส[3]*

โดยทั่วไปการป้องกันโรค *มัยโคพลาสมา กัลลิเซพติกุม* ในไก่ไข่จะทำโดยการให้วัคซีนชนิดรีคอมบิแนนท์ ไวรัสฝีดาษไก่-*มัยโคพลาสมา กัลลิเซพติกุม* (อาร์เอฟพีวี-เอ็มจี) หรือให้เชื้อเป็นสายพันธุ์เอฟ (เอฟเอ็มจี) อย่างใดอย่างหนึ่ง การศึกษานี้มีวัตถุประสงค์เพื่อประเมินการป้องกันโรคเอ็มจีในไก่ไข่ที่ได้รับวัคซีนอาร์เอฟพีวี-เอ็มจีและเอฟเอ็มจี เปรียบเทียบกับไก่ไข่ที่ได้รับวัคซีนเอฟเอ็มจีอย่างเดียว แบ่งไก่ไข่จำนวน 100 ตัว อายุ 8 สัปดาห์เป็น 4 กลุ่มดังนี้ กลุ่มที่ 1 ได้รับวัคซีนเอฟเอ็มจีเมื่ออายุ 11 สัปดาห์ กลุ่มที่ 2 ได้รับวัคซีนอาร์เอฟพีวี-เอ็มจีเมื่ออายุ 8 สัปดาห์และวัคซีนเอฟเอ็มจีเมื่ออายุ 11 สัปดาห์ กลุ่มที่ 3 และ 4 เป็นกลุ่มควบคุมบวกและลบ ตามลำดับ เมื่ออายุ 14 สัปดาห์ ไก่กลุ่มที่ 1-3 ได้รับเชื้อพิษทับเอ็มจีสายพันธุ์ไทย การป้องกันโรคนั้นประเมินจากน้ำหนักตัวไก่ที่เพิ่มขึ้น รอยโรคของถุงลมช่องอกและท่อลม และการตรวจพบเชื้อเอ็มจี เก็บซีรัมไก่เมื่ออายุระหว่าง 8-18 สัปดาห์เพื่อตรวจหาการตอบสนองของแอนติบอดีต่อเชื้อเอ็มจี ทำการผ่าซากไก่กลุ่มละ 5 ตัวเมื่ออายุ 12-16 สัปดาห์เพื่อประเมินรอยโรคถุงลมช่องอกทางมหภาคและท่อลมทางจุลพยาธิ เมื่ออายุ 16 และ 17 สัปดาห์ ทำการแยกเชื้อเอ็มจีและวินิจฉัยสายพันธุ์ด้วยวิธีอาร์เอพีดี พีซีอาร์ การศึกษาพบว่าเมื่อเปรียบเทียบไก่ระหว่างอายุ 14-18 สัปดาห์ที่ได้รับเชื้อพิษทับ ไก่กลุ่มที่ได้รับวัคซีนอาร์เอฟพีวี-เอ็มจีและวัคซีนเอฟเอ็มจีมีน้ำหนักตัวเพิ่มขึ้นและสามารถป้องกันการยึดเกาะของเชื้อเอ็มจีที่เป็นเชื้อพิษทับได้ โดยสรุป ไก่ที่ได้รับวัคซีนอาร์เอฟพีวี-เอ็มจีและวัคซีนเอฟเอ็มจีให้คุณลักษณะที่ดีกว่าเมื่อเปรียบเทียบกับไก่อีกสองกลุ่มที่ได้รับเชื้อพิษทับ โปรแกรมการให้วัคซีนทั้ง 2 ชนิดสามารถนำมาใช้ป้องกันโรคเอ็มจีในท้องที่ที่มีการระบาดได้

**คำสำคัญ:** ไก่ไข่ วัคซีนเชื้อเป็น เอฟเอ็มจี *มัยโคพลาสมา กัลลิเซพติกุม* การป้องกันโรค วัคซีนอาร์เอฟพีวี-เอ็มจี

*[1]หน่วยวิจัยสุขภาพสัตว์ปีก ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพ 10330 ประเทศไทย*

*[2]บริษัท ซีวา แอนิมัล เฮลธ์ (ประเทศไทย) จำกัด กรุงเทพ 10400 ประเทศไทย*

*[3]บริษัท ซีวา แอนิมัล เฮลธ์ เอเชีย แปซิฟิก เซรังงอ ประเทศมาเลเซีย*

***ผู้รับผิดชอบบทความ** E-mail: somsak.pa@chula.ac.th*