Assessment of a fowl pox-vectored *Mycoplasma gallisepticum* vaccine in chickens

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ABSTRACT

Fowl pox-vectored Mycoplasma gallisepticum vaccine (FPVMGV) is a recombinant fowl pox virus vaccine expressing protective Mycoplasma gallisepticum antigens which could facilitate the prevention of both fowl pox virus and M. gallisepticum infections. The objective of the present study was to determine the efficacy of fowl pox-vectored Mycoplasma gallisepticum vaccine as compared with live attenuated fowl pox, live mycoplasma gallisepticum and inactivated mycoplasma gallisepticum vaccines. The FPVMGV was proved to be safe based on clinical findings and gross pathological lesions of air sacs and peritoneum of the inoculated experimental chickens. FPVMGV was proved to induce higher protection (93%) than that induced by the conventional live (86%) and inactivated MG (80%) vaccines, in addition the vaccine induced protection against the challenge with fowl pox virus (92%) less than that in case of live attenuated fowl pox vaccine. On the other hand, the available commercial ELISA kit for detection of MG antibodies failed to detect any seroconversion in chicken vaccinated with the Fowl pox-vectored Mycoplasma gallisepticum vaccine contrally to the results obtained in the other two conventional MG vaccines. In conclusion, the fowl pox-vectored Mycoplasma gallisepticum vaccine could be a good option for use in chicken to prevent FPV and M. gallisepticum infections.

Key words: fowl pox vector, *Mycoplasma gallisepticum,* vaccine safety, vaccine potency

INTRODUCTION

Fowl pox is one of the important viral diseases affecting susceptible chickens of all ages *(1)*. Chickens should be vaccinated with live chicken embryo or cell culture propagated fowl pox virus and pigeon pox virus of high immunogenicity and low pathogenicity. In high-risk areas, vaccination with an attenuated vaccine of cell-culture origin in the first few weeks of life and revaccination at 12-16 wk is often sufficient. Because the infection spreads slowly, vaccination is often useful in limiting spread in affected flocks if administered when <20% of the birds have lesions. Because passive immunity may interfere with multiplication of vaccine virus, progeny from recently vaccinated or recently infected flocks should be vaccinated only after passive immunity has declined.Vaccinated birds should be examined 1 week later for swelling and scab formation at the site of vaccination. Absence of scab indicates lack of potency of vaccine, or improper vaccination. The interest to use fowlpox virus as a vector for expression of foreign genes was possible with the knowledge gained from studies with vaccinia viru. Mycoplasma gallisepticum is the aetiologic agent of chronic respiratory disease in chickens and infectious sinusitis in turkeys *(2)*. Mycoplasma gallisepticum (MG) infection have also been called Chronic Respiratory Disease (CRD). With the increasing of stress and high rearing density, Mycoplasma gallisepticum infection have already been a major problem that affects the poultry industry through its increased morbidity *(3)*. Infection with Mycoplasma gallisepticum is spread by aerosol exposure and egg transmission ,consequently, it can be exist and spread in flock for a long time *(4)*. According to the serological investigation and statistics, the average infection rate in flocks were 50%-80%

, when catching this disease, the unhealthy chicken rate increased about 10%, egg production of layer dropped 10%-20% ,weight lost 38%.,the period of onset to market prolonged , feed conversion efficiency reduced 20%. The best solution for controlling this disease may reside in the development of safe and effective vaccines. Inactivated available vaccine or attenuated strains of MG are commonly used within the layer industry to control MG-induced mycoplasmosis. A lot of countries have taken importance to preventing this disease for many years, but a kind of reasonable preventive way have not been found until now. The development of molecular biology techniques have opened up a new path to prevent this disease. Vectormune FP-MG is a genetically engineered live FPV vaccine for use in chickens and turkeys. The FPV has been genetically modified to express key protective Mycoplasma gallisepticum antigens. Vectormune FPMG is indicated as an aid in the prevention of fowl pox and M. gallisepticum infection *(5)*.

So, the present study was conducted to determine the safety and the potency of the vaccine comparing with other field types of mycoplasma and pox vaccines.

MATERİAL AND METHOD

Expermintal Chickens

A total of three hundred and fifty (300) SPF white leghorn chickens of 8-weeks old of age kept under strict hygienic measure of rearing and feeding of which two hundred and fifty (250) birds were divided to five groups used for laboratory evaluation of the safety and potency of the tested vaccine, while the rest 50 SPF chicken were kept as unvaccinated group.

Vaccines used

Fowl pox-vectored Mycoplasma gallisepticum vaccine (Vectormune FP-MG), which is produced by Ceva Biomune Animal Health Company (Lenexa, KS). the vaccine was used via wing web administration.

Imported Inactivated Mycoplasma gallisepticum vaccine, dose of 0.5 ml was inoculated S/C.

Imported Live Mycoplasma gallisepticum F strain vaccine, was adminstered via eye drops (10^7 CFU / dose).

Imported Live Fowl Pox Virus vaccine. The vaccine was used via wing web administration ($10^{3.5}$ $TCID_{50}$/dose).

Eperimental design

Group I: Comprised fifty birds were received 10 field dose of tested vaccine to study the safety of fowl pox-vectored Mycoplasma gallisepticum vaccine (FPVMGV).

Group II: Seventy birds were vaccinated with a fowlpox-vectored MG vaccine.

Group III: Forty birds were vaccinated with a available MG live vaccine.

Group IV: Forty birds were vaccinated with available MG inactivated vaccine.

Group V: Fifty birds were vaccinated with available Live Fowl Pox vaccine.

Group VI: Fifty birds were kept as a non vaccinated group.

Cell Culture

Primary chicken embryo fibroblasts (CEF) were prepared from 10 day old SPF chicken embryo; that obtained from Kom Oshiem Farm, Fayoum, Egypt were used for titration of (FPVV) fowl pox virus vaccine,according to OIE Protocol.

Identification of Fowl poxvirus and Mycoplasma genes by using PCR

Extraction of DNA

For DNA extraction, the Vectormune FP-MG vaccine was processed for detection of the mycoplasma gene insert using Dneasy kit (Qiagen Company) following the manufacturer instructions.

Polymerase Chain Reaction (PCR)

PCR was conducted on the viral DNA of the recombinant pox virus using primer pair specific for mycoplasma *(6)* and Fowl pox virus *(7,8)* (as shown in (Table 1). The PCR reaction mixture of 25 µl was prepared as 12.5 µl of 5 x green master mix PCR buffer, 1 µl of the forward primer, 1 ul of the reverse primer, 5 µl of DNA templet and 5.5 µl of water. The PCR reaction condition for MG gene amplification was initial denaturation at 94°C/3 min, followed by 40 cycles: (94°C/30 sec, 55°C/ 30 sec., 72°C/60 sec.), and one cycle of final extension 72°C/5min and soak at 4°C. The program cycling of fowl pox gene was subjected to 32 cycles denaturation 5 min at 94°C; annealing 1 min at 60°C; extension 1 min at 72°C, in a programmable cyclic reactor. PCR products were detected by running in 1% Agarose gel electrophoresis incorporating 100bp marker followed by examination under U.V light.

Table 1. Primers sequencing

Primers	Nuclotide sequence	Size of product
MG	F:5'-AATCTTGGCATAGTCTTGTAGTGA-3' R: 5'-ATGATCTTAGAAACGCTGTAGTAG-3'	500
Fowl Pox virus-4b gene	P1,5'-CAGCAGGTGCTAAACAACAA-3' P2, 5'-CGGTAGCTTAACGCCGAATA-3'	578

Safety Test

The safety of fowl pox-vectored Mycoplasma gallisepticum vaccine was carried out using 10 field dose of the tested vaccine. Fifty bird of group I inoculated by wing web and the birds are observed for 7–10 days for evidence of pocks on unfeathered area for fowl pox virus. Chicken were observed for clinical signs of mycoplasma gallisepticum icluding tracheal rales, nasal discharge and coughing, and for the absence of adverse effects attributable to the vaccine.

Serological test

Serum samples were collected weekly from different vaccinated bird groups either received mycoplasma vaccines, live pox vaccine or vectormune FP-MG vaccine.

Enzyme Linked Immunosorbent Assay (ELISA) test was carried out to detect humoral antibodies to fowlpox virus according to *(9)* and the result was calculated as S/P ratio:

$$\text{S/P ratio} = \frac{\text{Tested sample mean} - \text{Negative sample mean}}{\text{Positive sample mean} - \text{Negative sample mean}}$$

And for Mycoplasma gallisepticum using ELISA kit obtained from Biocheck Poultry Immunoassay for detection of Mycoplasma specific antibodies.

Challenge tests

Thirty chickens from each vaccinated group and 20 chicken from unvaccinated group were challenge 3 weeks post vaccination using Mycoplasma galliseplicam strain (virulent R.strain) which was obtained from the Central Lab. For Evaluation of Vet. Biologics, Abbasia, Cairo. 0.1 ml of an overnight culture containing (3.3 x 10^6 CFU/ml) were inoculated into the posterior thoracic air sac. All birds are necropsied 7–14 days post-challenge, and air sac lesions are scored.

Virulent fowl pox virus was supplied by the Central Lab. For Evaluation of Vet. Biologics, Abbasia, Cairo. All groups of chickens including unvaccinated group were challenged with 10^3 EID_{50}/50 µl of virulent FPV through wing-web puncture. The birds are observed for 7–10 days for evaluate the evidence of takes.

All of the chickens after challenge exposure, were kept in a separate isolator and observed up

to 21 days for the development of clinical signs and symptoms.

RESULTS AND DİSSCUSİON

Concerning the fowl pox-vectored Mycoplasma gallisepticum vaccine, the most important point was proved to be containing MG insert gene, as detected by PCR at the molecular weigt 500 bp mean while , the obtained PCR product for the fowl pox virus was at the molecular weight 578 bp as shown in Fig. (1 & 2).

The fowl pox-vectored MG virus vaccine titre was $10^{3.8}$ TCID/dose.

Fowl pox virus is the best-studied member and type species of the Avipox virus genus of the Poxviridae. Fowl pox virus has been suggested as a possible vector for vaccines use not only in poultry but also in mammals including humans *(10-12)*. Several important poultry pathogens became early targets for FPV vector vaccines, especially avian influenza virus, Newcastle disease virus, Marek's disease virus, infectious bronchitis virus, and infectious bursal disease virus *(13-15))*.

MG is the most pathogenic and economically significant mycoplasma pathogen of poultry due to Economic losses from condemnation, downgrading of carcasses, reduced feed and egg production, and increased medication costs *(16)*. Avian mycoplasma is controled with different types of vaccines. MG vaccines have been used in the control of MG in areas where eradication is not feasible. MG vaccines are used to prevent or reduce disease and clinical signs in the vaccinated birds as well as to prevent egg production losses and egg transmission of MG. Inactivated MG vaccines have been widely used in several countries.

The results with MG bacterins have been variable; some investigators found that bacterins could protect broilers from airsacculitis *(17)* and layers from reduction in egg production *(18)* while others did not detect much efficiency in commercial egg layers with endemic MG infection.

Mycoplasma gallisepticum live vaccines is an efficacious approach to reduce susceptibility to infection and to prevent the economic losses. An important characteristic of MG live vaccines is their ability to induce resistance to wild-type strain infection, and to displace wild-type strains with the vaccine strain on multiple-age production sites *(19)*.

The increased use of live MG vaccines requires more powerful tools to trace the source of infection and to differentiate vaccine strains from circulating field isolates. Vectormune FP-MG which is vector vaccine have more benefit and safety in chicken *(5)*.

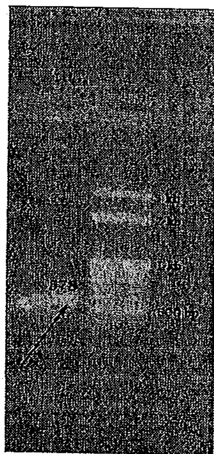

Fig.1. Amplification of the 500 bp fragment of MG gene
Lane 1: MG gene,
Lane 2: 100 base paire lader marker

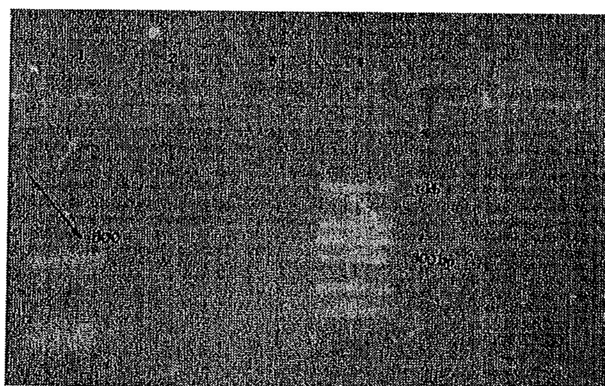

Fig. 2. Amplification of the 578 bp fragment of Fowl pox virus gene
Lane 1:Positive PCR product of Fowl pox virus
Lane 4: 100 bp ladder Marker

According to OIE 2013 *(20),* the safety test of the fowl pox-vectored MG virus vaccine was satisfactory when the injected bird with ten field dose through wing web in the SPF chickens and showed no death or disease symptoms.

Humoral antibody response was evaluated by detecting the ELISA antibodies titre as shown in table 2 and 3 and the potency test as shown in Table 4 and 5.

serum samples were collected weekly for detection of M. gallisepticum and fowl pox antibodies by ELISA test from all vaccinated groups . It was found that the serum samples of mycoplasma gallisepticum were positive in group III and group IV and show no MG antibodies titer in the group II which was vaccinated with fowl pox-vectored Mycoplasma gallisepticum vaccine and that similar with some previous studies *(21,22).*

In addition to the above, the results recorded in table 2 and 5, we noticed that The fowl pox antibodies titer appeared from the 1st week of vaccination and increase till reached the to the peak at 4th week post vaccination, it reached to 1.84575 and 1.7202

in the vaccinated chicken in group II and group V respectively. There is high signficant antibodies titer in group II which was vaccinated with fowl pox-vectored Mycoplasma gallisepticum vaccine. The fowl pox protection percentage was 92% and 100 % in fowl pox-vectored Mycoplasma gallisepticum vaccine and live fowl pox vaccine respectively.

Air-sac lesion scoring was used for record of mycoplasma protection. the MG protection percentage was 93%, 86% and 80% in fowl pox-vectored Mycoplasma gallisepticum vaccine, live mycoplasma vaccine and mycoplasma gallisepticum inactivated vaccine respectively.

Table 2.Mean of Fowl pox ELISA antibody titer in chickens vaccinated with Vectormune FP-MG vaccine and live fowl pox vaccine

Weeks post vaccination	F1	F2
1st week	0.832625	0.723956
2nd week	1.183875	1.05435
3rd week	1.505625	1.3366
4th week	1.84575	1.7202
5th week	1.52225	1.483076
6th week	1.45625	1.410429
7th week	1.216125	1.14612
8th week	0.839375	0.4437

F1: Fowl poxs EIISA titre of Vectormune FP-MG vaccine F2: Fowl poxs EIISA titre of Live fowl pox vaccine

Table 3. Mean of MG ELISA antibody titer in chickens vaccinated with Vectormune FP-MG vaccine, Live MG vaccine and inactivated MG vaccine

Weeks post vaccination	M1	M2	M3
1st week	0.029	0.98	0.76
2nd week	0.021	2.08	3.01
3rd week	0.028	2.86	3.345

M1: MG EIISA titre of Vectormune FP-MG vaccine
M2: MG EIISA titre of Live MG vaccine
M3: MG EIISA titre of inactivated MG vaccine.

Table 4. Protection Percentages of fowl pox in chickens vaccinated with Vectormune FP-MG vaccine and live fowl pox vaccine

Challenge time post vaccination	Chicks group	No. of challenged Chickens/group	No. of birds showing lesion post challenge			Protection percent (%)
			5dpc	7dpc	10dpc	
10 days post vaccination	chickens vaccinated with fowl pox vectored *Mycoplasma gallisepticum* vaccine **(G: II)**	25	1	1	0	92%
	chickens vaccinated with live fowl pox vaccine **(G: V)**	25	0	0	0	100%
	Control unvacinated SPF chicken **(G:VI)**	20	5	10	20	0%

G II: chickens vaccinated with Vectormune FP-MG vaccine
G V: chickens vaccinated with live fowl pox vaccine
G VI: control unvaccinated SPF

Table 5. Protection percenteg of MG in chickens vaccinated with Vectormune FP-MG vaccine, Live MG vaccine and inactivated MG vaccine

Challenge time post vaccination	Chicks group	No. of challenged Chickens/ group	No. of birds showing air saculitis post challenge		Protection percent (%)
			7 dpc	14 dpc	
3 weeks post vaccination	chickens vaccinated with Vectormune FP-MG vaccine **(G: II)**	30	0 0	2	93%
	chickens vaccinated with live MG vaccine **(G: III)**	30	2	2	86.6 %
	chickens vaccinated with inactivated MG vaccine **(G: IV)**	30	4	2	80%
	Control unvaccinated SPF chicken **(G: VI)**	20	10	7	15%

G II: chickens vaccinated with Vectormune FP-MG vaccine
G III: chickens vaccinated with live MG vaccine
G IV: chickens vaccinated with inactivated MG vaccine
G VI: control unvaccinated SPF

In our investigation, it could be concluded that the comparing of the conventional vaccines against fowl pox virus and mycoplasma gallisepticum with the fowl pox-vectored Mycoplasma gallisepticum vaccine was found that the fowl pox-vectored Mycoplasma gallisepticum vaccine have more benefits and showed more safety profile in SPF chickens. This study demonstrates that vaccination with Fowl pox-vectored Mycoplasma gallisepticum vaccine have no adverse vaccine reactions or clinical signs were observed, and all of the chickens remained clinically healthy. Also the results suggest that the protection to MG following obtained by the Fowl pox-vectored Mycoplasma gallisepticum vaccine were highly satisfactory.

REFERENCE

1.*Fenner, Frank J, Gibbs E Paul J, Murphy Frederick A, Rott Rudolph Studdert, Michael J, White David O (1993).* Veterinary Virology (2nd ed.). Academic Press, Inc. ISBN 0-12-253056-X

2.*Shil NK, Rahman MS, P aul S, Cha SY, Jang HK, Song HJ (2007):* Vaccination Studies against Fowl Pox in Chickens. Korean Journal of Poultry Science 34:4, 253-257

3.*Jordan F TW (1979):* Avian mycoplasmas, p. 9–16. In J. G. Tully and R. F. Whitcomb (ed.), The mycoplasmas, vol. 2. Academic Press,New York, N.Y.

4. *Soeripto KG, Whithear GS, Cottew and Harrigan KE (1989):* Virulence and transmissibility of Mycoplasma gallisepticum. Aust. Vet. J. 66:65–72.

5. *Zhang GZ, Zhang R, Zhao HL,Wang XT, Zhang SP, Li, XJ, Qin, CZ, Lv, CM, Zhao, J X and Zhou JF (2010):* Poultry Science 89 :1301–1306 doi: 10.3382/ps.2009-00447.

6. **Biomune Co US Vet. Lic. No. 368.2000.**

7. *Binns, MM, Boursnell, MEG, Tomley, FM and Campbell J (1989):* Analysis of the fowlpoxvirus gene encoding the 4b core polypeptide and demonstration that it possesses efficient promoter sequences. Virology 170, 2888291.

8.*Long Huw Lee and Kuo Hwa Lee (1997):* Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. Journal of Virological Methods 63: 113- 119

9. *Buscaglia C, Bankowski RA and Miers L (1985):* Cell-culture virus neutralization test and enzyme-linked immunosorbent assay for evaluation of immunity in chickens against fowlpox. Avian Dis., 29, 672–680.

10.*Boursnell ME, Green PF, Campbell JI, Deuter A, Peters RW, Tomley FM, Samson AC, Chambers P, Emmerson PT and Binns MM (1990):* Insertion of the fusion gene from Newcastle disease virus into a non-essential region in the terminal repeats of fowlpox virus and demonstration of protective immunity induced by the recombinant. J. Gen. Virol. 71:621-628..

11. *Skinner MA, Laidlaw SM, Eldaghayes I, Kaiser P and Cottingham MG (2005):* Fowlpox virus as a recombinant vaccine vector for use in mammals and poultry. Expert Rev. Vaccines 4:63–76

12.*Pozzi E, Basavecchia V, Zanotto C, Pacchioni S, Cde G Morghen and Radaelli A (2009):* Construction and characterization of recombinant fowlpox viruses expressing human papilloma virus E6 and E7 oncoproteins. J. Virol. Methods 158:184–189.

13.*Swayne, DE, Garcia M, Beck JR, Kinney N and Suarez DL (2000):* Protection against diverse highly pathogenic H5 avian influenza viruses in chickens immunized with a recombinant fowlpox vaccine containing an H5 avian influenza hemagglutinin gene insert. Vaccine 18:1088–1095.

14.*Lee LE, Witter RL, Reddy SM, Wu P, Yanagida N and Yoshida S (2003):* Protection and synergism by recombinant fowl pox vaccines expressing multiple genes from Marek's disease virus. Avian Dis. 47:549–558.

15.*Davison S, Gingerich EN, Casavant S and Eckroade RJ (2006):* Evaluation of the efficacy of a live fowlpox-vectored infectious laryngotracheitis/avian encephalomyelitis vaccine against ILT viral challenge. Avian Dis. 50:50–54.

16.*Ley HD (2003):*Mycoplasma gallisepticum Infection. Introduction. In: Diseases of Poultry, 11th ed. D. E. Swayne, eds. Iowa State University Press, Ames, Iowa. pp. 722.

17.*Yoder HW Jr, Hopkins SR and Mitchell BW (1984):* Evaluation of inactivated Mycoplasma gallisepticum oil-emulsion bacterins for protection against airsacculitis in broilers. Avian Dis. 28:224-234.

18.*Hildebrand DG, Page DE and Berg JR (1983):* Mycoplasma gallisepticum (MG) laboratory and field studies evaluating the safety and efficacy of an inactivated MG bacterin. Avian Dis. 27:792-802.

19.*Levisohn S and Kleven SH (2000):* Avian mycoplasmosis (Mycoplasma gallisepticum). Rev. Sci. Tech. 19:425-442.

20. *OIE, Manual of Diagnostic Tests and Vaccines for Terrestrial Manual, (2013).*

21. *Whithear KG, Soeripto, KE Harrigan and Ghiocas E (1990)*: Immunogenicity of a temperature sensitive mutant Mycoplasma gallisepticum vaccine. Aust. Vet. J. 67:168–174.

22.*Noormohammadi AH, Jones JE, Underwood G and Whithear KG (2002)*: Poor systemic antibody response after vaccination of commercial broiler breeders with Mycoplasma gallisepticum vaccine ts-11 not associated with susceptibility to challenge. Avian Dis. 46:623–628.

الملخص العربى

تقييم لقاح جدري الطيور والميكوبلازما المهندس وراثيا في الدجاج

عفاف احمد خضر، هيام فاروق
المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية – العباسية – القاهرة

يعتبر لقاح جدري الطيور المحمل عليه الميكوبلازما لقاح مهندساً وراثياً لفيروس جدري الطيور المحمل عليه جين الميكوبلازما جاليسيبتكم والذى يمكن أن يستخدم في الوقاية من فيروس جدري الطيور وميكروب الميكوبلازما جاليسيبتكم. وكان الهدف من هذه الدراسة هو تحديد مدى فعالية لقاح جدري الطيور والميكوبلازما جاليسيبتكم المهندس وراثيا بالمقارنة بلقاح جدري الطيور المستضعف الحي، ولقاح الميكوبلازما الحي ولقاح الميكوبلازما المثبط. وقد تم التأكد من أن لقاح جدري الطيور والميكوبلازما المهندس وراثيا آمن اعتماد علي النتائج الإكلينيكية والباثولوجية للاكياس الهوائية و الغشاء البريتونى في دجاج التجربة المحقون باللقاح. وفيما يتعلق بالكفاءة، فقد وجد ان لقاح جدري الطيور المحمل عليه الميكوبلازما جاليسيبتكم يعطي نسبة حماية ضد ميكروب الميكوبلازما جاليسيبتكم (٩٣%) أعلى من مثيلتها في لقاح الميكوبلازما الحي (٨٦%) ولقاح الميكوبلازما المثبط (٨٠%). بينما أعطى اللقاح نسبة حماية ضد فيروس جدري الطيور(٩٢%) اقل من مثيلتها في لقاح جدري الطيور المستضعف الحي (١٠٠%). على الجانب الاخر، فشلت كيت الإليزا التجارية في الكشف عن الأجسام المناعية ضد ميكروب الميكوبلازما جاليسيبتكم في مجموعة الدجاج المحصن بلقاح جدري الطيور والميكوبلازما المهندس وراثيا، مخالفا للنتائج الاليزا في اللقاحات الأخرى. وفي النهاية يمكن أن يكون لقاح جدري الطيور المحمل عليه الميكوبلازما خيارا جيدا للاستخدام في تحصين الدجاج لمنع الإصابة بمرضي جدري الطيور والميكوبلازما جاليسيبتكم.