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TRIAL FOR PREPARATION AND EVALUATION OF INACTIVATED MYCOPLASMA GALLISEPTICUM VACCINE IN BROILERS

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SUMMARY

An inactivated Mycoplasma gallisepticum (MG) vaccine was prepared using R- Strain of MG. The vaccine was tested for safety, sterility and purity. Ten thousands, 10- day - old broilers were randomly divided into two equal groups (A and B). Group A was given 0.5 ml of a saponin inactivated aluminum hydroxide gel adjuvant MG vaccine subcutaneously, while group B was kept as control unvaccinated. vaccinated birds (group A) exhibited good levels of MG antibodies, in addition to an increase in body weight , feed conversion rate and low mortality, compared with birds in group B (unvaccinated).

80 chicks (40 from group A and 40 from group B) were moved to an isolated site and challenged with virulent R. strain of MG four weeks post vaccination. Birds from group A revealed decrease in air sac lesion scores and increased MG antibodies up to six weeks post vaccination when compared to Group B.

INTRODUCTION

Mycoplasma gallisepticum (MG) is the primary etiological agent responsible for chronic respiratory disease(CRD)in chickens resulting in substational economic losses to poultry producers throughout the world (Ley and yoder, 1997).

Attempts to control mycoplasmosis and to mitigate the effects of the disease depend on intensive and prolonged use of antibiotic medication in both prophylactic and therapeutic doses. yet such therapy does not result in clearance of the Mycoplasmas. Moreover, antibiotic-resistant strains have been found and the expense associated with treatment is often prohibitive (Hildebrand et al.. 1983)

Currently, inactivated and live vaccines are available to poultry farms. Although inactivated vaccines were not well accepted in the past, they are often preferred today mainly because there is no risk of infection and because they don't affect Mycoplasma gallisepticum detection (Nascimento et al.,2005).

Live Mycoplasma gallisepticum vaccines may be pathogenic and lead to MG outbreak (F-vaccine) in young chicks (Rodriguez and Kleven,1980) and turkeys (Lin and kleven, 1984) and others (Ts-11and 6/85) give low level of protection (Abd-El-Motelib and kleven, 1993).

Vaccination with MG bacterins has been shown to reduce but usually not eliminate colonization by MG following challenge (Yaghashi et al., 1992)

Various adjuvant and antigen delivery systems had been used to enhance the performance of non-living MG vaccines (Elfaki et al., 1993).

Recently novel in activators such as saponin and binary ethylene imine (BEL) have been proven to be efficient in maintaining the antigenic properties of the pathogen. This ensures their identification by the host immune system and hence enhancing a protective immune response. The unencouraging results of inactivated MG preparations reflected either the use of inadequate antigen concentration and potency or either the use of in effective adjuvant (Amal Ragab, 2006).

Although the vaccination of parent flock resulted in high haem-agglutination inhibition titer in the progeny, optimal and constant protection could be attained by their vaccination (Bayoumi et al., 1988). Maternal antibodies in chicks originated from immune breeders conferr very little protection against aerosol challenge with virulent R strain of MG (Lin and Kleven, 1984)

The aim of this study was to prepare and evaluate the safety and efficiency of a saponin inactivated aluminum hydroxide gel adjuvant MG vaccine in broilers under field circumstances.

MATERIALS AND METHODS

1-Mycoplasma gallisepticum-R strain vaccine preparation (MG-R):

The R strain of MG was kindly supplied by Dr. Kleven S.H. (Department of Avian Medicine, University of Georgia, Athens, Georgia). It was grown in Freys medium (Frey et al., 1968)/24 hours / 37°C, then harvested and the deposited cells were re-suspended in 0.01 M phosphate, buffer saline (pH 7.2) to give cell concentration of I x 10^{10} color changing unit (CCU/ml) just prior to inactivation with saponin using the method described by Yoder and Hopkins (1985).

Aluminum hydroxide gel vaccine (Honil Limited, London, UK) was prepared by adding one volume

of the gel to four volumes of the concentrated antigen according to Salt et al.(1994), using a magnetic stirrer at approximately 300 rpm.

2- Quality control testing of saponin inactivated aluminum hydroxide gel adjuvant MG-R vaccine:

The aforementioned vaccine was subjected to purity, sterility, safety and potency criteria according to OIE (2000).

3- Field evaluation of the prepared MG vaccine:

(a) Potency

Ten thousand commercial broilers, 10-days old and susceptible to MG infection were divided into 2 groups, 5000 of each. The first group was inoculated subcutaneously in the middle of the neck with 0.5 ml of a saponin inactivated aluminum hydroxide gel adjuvant MG-R vaccine. The second group was held as an unvaccinated control one.

(b) Challenge:

Fourty chickens of each group, were, moved to floor pens in isolated sites and four weeks post vaccination were challenged with 0.1 ml of an overnight virulent MG culture (R strain) containing 3.8 x 10^6 CFU/ ml according to Loughnane et al.(1993). Blood samples were collected weekly till 6 weeks post vaccination The serum samples were subjected to both serum plate agglutination test (SPA) according to Kempf et al. (1997) and Enzyme Linked Immunosorbent Assay (ELISA) (KPL ELISA Kit).

Both groups were kept under observation. Birds were euthanatized and weekly examined for air-sac lesions post challenge and scored from 0 (no lesion), to 4 (sever lesions) as described by Abd-El-Motelib and Kleven (1993) for 5 weeks according to Yoder, (1986).

Air sac swabs were taken from necropsied chickens. These were streaked directly onto agar plates which were then incubated at 37°C for 7 days to isolate mycoplasma.

RESULTS

Characteristic colonies were obtained when the antigen concentrates was tested on the mycoplasma agar media indicating that only R-strain of MG-was present. The prepared vaccine batch was found to be free from any contamination by aerobic, anaerobic bacterial or fungal contamination.

The obtained results in table (1) showed that the prepared vaccine was safe when it induced no clinical signs or air sac lesions or other PM lesions in vaccinated birds.

Table (1): Results of safety described as clinical signs and air sac lesions in chickens 3 weeks post inoculation.

Chicken groups	No. of tested birds	No. of birds showing Clinical sings			No. of birds showing air sac lesions		
		Positive/tested	%of positive	Safety %	Positive/tested	%of positive	Safety %
Vaccinated	10	0/10	0%	100%	0/10	0%	100%
Control	10	0/10	0%	100%	0/10	0%	100%

By the beginning of the 4th week of age, the unvaccinated birds in group B began to have some respiratory signs in the form of tracheal rales, conjunctivitis, lacrimation, nasal discharge, coughing, inappetence and loss weight .Also some birds in the flock had swelling of the facial skin. On the other hand birds in group A exhibited mild respiratory signs. Gross lesions of birds in group B showed catarrhal exudate in nasal passage airsacs frequently contained caseous exudate. Some of the dead birds had some degrees of pneumonia.

The vaccinated chicken in group A showed a feed conversion rate better than the non vaccinated Group B indicated as an increase in the average mean body weight in the vaccinated group compared with the unvaccinated chicken group as shown in table (2).

The results described in table (3) indicated that the serum of vaccinated chicken tested with SPA test showed positive agglutination as early as one week post vaccination in 25% of the tested samples and increased gradually up to 62.25 %,75% and 100% at 2, 3 and 4 weeks post vaccination, respectively.

Table (2): Consumed ration, mean body weight, mortality and under weight birds of broilers vaccinated and unvaccinated with saponin inactivated aluminum hydroxide adjuvant *MG*-R vaccine.

	No	Species	Consumed feed/ bird through 45 days	Mean of b. wt at age 45days	Mortality through 45 days	Mortality Percentage %	No of under weight birds	under weight percentage %
Vaccinated group	5000	Hubbered	3.9kg	2.158kg	205	4.1%	163	3.2
Non vaccinated group	5000	Hubbered	3.8kg	1.940	269	5.3%	253	5.6

Table (3): Results of SPA test carried out on serum samples of vaccinated chickens with the prepared inactivated *MG-R* vaccine.

Weeks post vaccination	Vaccinated group		Control	group
	Positive/tested	% of positive	Positive/tested	% of positive
I	10/40	25%	0/40	0 %
2	25/40	62.5%	0/40	0 %
3	30/40	75%	0/40	0 %
4	40/40	100%	0/40	0 %
5	40/40	100%	0/40	0 %
6	40/40	100%	0/40	0 %

Table (4): Mean titer of ELISA of the serum samples of vaccinated chickens with the prepared *MG* vaccine.

Weeks post vaccination	Vaccinated group				Control group			
	No. of tested birds	No. of positive	ELISA mean titer	% of positive	No. of tested bird	No. of positive	ELISA mean titer	%of positive
1	40	15	0.296	37.5%	20	0	0.205	0%
2	40	30	0.298	75%	20	0	0.198	0%
3	40	35	0.311	87.5%	20	0	0.182	0%
4	40	40	0.329	100%	20	0	0.145	0%
5	40	40	0.347	100%	20	0	0.149	0%
6	40	40	0.345	100%	20	0	0.159	0%

Concerning the positive EL1SA titer in table (4), it was noticed that a titer was obtained as early as the first week post vaccination in 37.5% of tested samples raised to 75% at the second week post vaccination to increase to 87.5%, 100%, 100%, and 100% at third, fourth, fifth and six week post vaccination, respectively.

As regard to the air sac lesion scores in the vaccinated chickens after challenge with virulent R-strain, (Table 5), it appeared that the vaccinated groups showed marked and significant lower scores than the control unvaccinated chicken group starting one week post challenge up to the fifth week post challenge.

Table (5): Air sac lesions scores in chickens vaccinated with prepared inactivated *MG* vaccine and challenged with virulent *MG-R* strain.

Chicken group	Air sac lesion scores/Weeks post challenge				
	1	2	3	4	5
Vaccinated	0.39	0.81	1.18	1.27	1.29
Control	1.14	1.97	2.96	3.27	3.54

DISCUSSION

Hayatsu et al (1975) mentioned that protection against respiratory tract infection could not be relatively obtained but protection against the air sacculitis was possible. Also Lin and Kleven (1984) have suggested that the level of protection obtained with live MC-vaccines correlates with the virulence of the vaccine strain. So the present study, was directed to prepare an inactivated vaccine using saponin to increase the potency of the vaccine. Our findings showed that the vaccinated chickens had a body weight gain higher (2.108 kg) than the unvaccinated chickens (1.940 kg) and this reflected a better feed conversion rate in the vaccinated group. These findings agree with Bayoumi et al., (1988).

Birds in group B had clinical signs in the form of tracheal rales conjunctivitis, lacrimation, nasal discharge, coughing and in appetence which were consistent with Saif, et al.,(2005). Caseous air sacculilits usually seen in dead birds from group B in addition to catarrhal exudate in nasal passages and trachea. These signs were either absent in most of the birds or noticed in a few birds in mild form in Group A.

The vaccinated chicks had a low mortality rate and low number of under weight birds (205 and 163 respectively) compared with unvaccinated control (269 and 253 respectively) as seen in table (2)

It is revealed that, the serological tests (SPA or ELISA) showed protective antibody titer reaching its maximum (100%) level at the fourth week post vaccination and continued to six weeks post vaccination in spite of the protective titer appeared as early as the first week post vaccination in 25 % and 37.5% of the tested samples when tested with SPA and ELISA respectively. These levels of antibody correlated with the results obtained after challenge with virulent strain of MG appeared as different grads of air saculitis depending upon the immune status of the birds. On the other hand the unvaccinated chickens showed high grades of air saculitis in comparison with the vaccinated group and this inconsistent with Yoder (1986) &Yoder et al. (1984).

It was observed that, vaccination with MG- inactivated vaccine prepared from R strain of MG and challenged with a virulent culture of MG-evoked a high degree of protection in vaccinated chickens. Also Sundquist et al. (1996), reported that. for a saponin inactivated vaccine, an experimental immunostimulating complex vaccine of M. gallisepticum induced protective immunity and signif icantly reduced lesions in the air sacs after chal lenge with virulent MG-strains. Moreover (Am: Ragab, 2006) concluded that MG saponin inact vated vaccine was better than the MG-F live va cine and MG formalin inactivated.

As maternal antibodies in chicks originated fro

immune breeders conferred very little protection against aerosol challenge with virulent R strain of MG (Lin and Kleven, 1984), schedules for vaccination of broilers with inactivated bacterins must be prepared.

It can be concluded that, the prepared vaccine is of value because it gave acceptable protection level in vaccinated chickens and potentiate the feed conversion and body weight gain in comparison with the unvaccinated chickens, and it could be recommended that the production of this vaccine can be used to avoid the CRD and its complication problems in the field.

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محاولة لتحضير وتقييم لقاح مثبط لمرض الميكوبلازما جالليسيتكم فى بدارى التسمين

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**المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية القاهرة
***كلية الطب البيطرى – جامعة بنها – قسم أمراض الدواجن

فى هذه الدراسة تم تحضير لقاح مثبط للميكوبلازما جالليسبتكم من العترة R وتم إختبارة من ناحية النقاوة والأمان والكفاءة المناعية .

تم تقسيم ١٠ آلاف بدارى تسمين عمر ١٠ أيام عشوائياً إلى مجموعتين ثم تحصين المجموعة الأولى (A) باللقاح المحضر و المجموعة الأخرى (B) تم الإحتفاظ بها كضوابط غير محصنة للتجربة .

المجموعة المحصنة (A) أعطت مستوى مناعى جيد من الأجسام المضادة أكثر من ٦ أسابيع بعد التحصين للميكوبلازما جالليسبتكم و زيادة فى وزن الطيور المكتسب وزيادة فى معدل التحويل الغذائى كما أنها أقل فى نسبة الوفيات مقارنة بالمجموعة (B) (ضوابط التجربة) .

تم إجراء إختبار التحدى بإستخدام العترة الضاريه R للميكوبلازما جالليسبتكم بعد ٤ أسابيع من التحصين لمجموعة من بدارى التسمين فى كلا المجموعتين .

الطيور من المجموعة الأولى (A) كانت أقل بكثير فى معدل الإصابة بالأكياس الهوائية عن المجموعة الثانية (B) بالإضافة إلى مستوى مناعى عالى عالى حتى ٦ أسابيع بعد التحصين .