Preparation Of New *Mycoplasma Gallisepticum* Live Vaccines And Their Evaluation In Chickens

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ABSTRACT

This study comprised the preparation of local live *Mycoplasma gallisepticum (MG)* vaccines (ts-11 and 6/85 strains), and their evaluation in comparison with F strain vaccine in chickens through laboratory experiment, using PCR and RAPD techniques for the detection of *Mycoplasma* vaccine strains. Eighty, day-old *Mycoplasma* free chicks (Avian 48 breed) were divided into eight groups, subjected to blood and tracheal swabs at the first, till sixth week post-vaccination. Serum plate agglutination (SPA) test showed the highest positive *MG* antibodies in F strain vaccinated and the control positive groups. Followed by ts-11, 6/85 vaccinated and contact groups, and F strain contact group. Control positive group showed the highest geometric mean titer (1054.32) by ELISA, followed by F strain vaccinated and its contact (932.03 and 818.81, respectively). While ts-11 and 6/85 vaccinated and contact groups were suspected (734.43, 703.04, 673.62 and 525.78, respectively). *MG* could be isolated from 100% of the control positive and F strain vaccinated group and increased from 50-90% in the F strain contact group. Isolation rate increased to 80% in ts-11 vaccinated group, but it was only 50% in its contact one. In 6/85 vaccinated and contact groups, the recovery rate was (60% and 50%, respectively).Polymerase chain reaction (PCR) detected 90-100% *MG* positive isolates percent in F strain vaccinated and control positive groups. While in F strain contact group, the positive percent ranged from 50-90%.*MG*-PCR detected 60-80% positive isolates percent in ts-11 vaccinated group and 0-50% in its contact one. In 6/85 vaccinated group, *MG*-PCR detected 30-60% positive isolates percent and 0-50% in its contact group. Random amplified polymorphic DNA (RAPD) technique confirmed the presence of F, ts-11and 6/85 strains in vaccinated and contact groups. The F strain vaccine provided good immunity against *MG* challenge. The ts-11 strain induced a milder post vaccination protection than F strain. DNA patterns of *MG* strains isolated from 6/85 strain vaccinated and contact groups were more similar to those isolated from *MG* challenge strain. Therefore, 6/85 strain could not provide good protection against *MG* virulent strain.

INTRODUCTION

MG infection in chickens is characterized by respiratory signs in the form of rales, coughing, nasal discharge and air sacculitis *(1)*. The need for producing safe, effective *MG* vaccines and their ability to elicit a protective immune response must be considered. A temperature sensitive *MG* strain known as ts-11 was produced by mutagenesis of the Australian field isolate 80083 H *(2)*. It failed to cause gross lesions or loss in egg production, whereas the parent 80038H strain did *(3)*. From the safety point of view, ts-11 was potentially useful vaccine strain. Although ts-11 vaccine produced low level of serum antibodies but vaccinated birds were usually able to resist direct challenge into the air sacs with large doses of virulent *MG*.

6/85 strain has been shown to be a safe and effective vaccine against *MG* for chickens. Safety was evaluated by observing absence of increase in air sac lesions *(4)* and no effects on egg production, egg size, and egg quality parameters *(5)*. Vaccinated chickens may

exhibit a poor to negative serological response *(6)*, but protection is still evident *(4)*.

MATERIAL AND METHODS

1) Experiment design: Eighty, day-old *Mycoplasma* free avian 48 chicks were used in the experiment. These chicks were subjected to PCR test to be sure that they are *Mycoplasma* free with continuous monitoring by PCR test till 28 day old (age of vaccination). Three vaccines were used (F strain, ts-11 strain, and 6/85 strain vaccines) in vaccination of chickens against mycoplasmosis. The design of the experiment is summarized in Table (1).
2) Media used for preparation of vaccines were as previously described *(7)*.
3) Vaccines were prepared as described by ***Lin and Kelren (8)***.
4) ELISA assay was applied using *MG* antibody kits *(9)*: Kirkeguard and Perry Lab (KPL).
5) Polymerase chain reaction technique was carried out as previously described *(10)*.
6) Random amplified polymorphic DNA (RAPD) technique was used *(11)*.

Table 1. Design for experimental evaluation of new living MG vaccines in avian 48 chickens

Group	No. of Birds	Classification of Birds	Dose of vaccination	Route of Administration	Age of birds at vaccination
1	10	F strain vaccinated group	0.1 ml	Intranasal	28 day
2	10	Contact of F strain vaccinated group	-	-	
3	10	ts-11 strain vaccinated group	0.1 ml	Intranasal	28 day
4	10	Contact of ts-11 strain vaccinated group	-	-	
5	10	6/85 strain vaccinated group	0.1 ml	Intranasal	28 day
6	10	Contact of 6/85 strain vaccinated group	-	-	
7	10	Positive control	0.1ml	Intranasal	28 day
8	10	Negative control	-	-	28 day

RESULTS

1.Serological evaluation of MG live vaccines

1.1.Serum plats agglutination (SPA) Results

Table 2 showed the SPA results for the detection of *MG* antibodies in all groups. In F strain vaccinated group, the SPA positive percent was increasing till reached 100%, while in its contact group, the SPA positive percent increased then decreased at the end of the experiment to reach 80%. ts-11 and 6/85 strains vaccinated groups and their contact ones, the SPA positive percent increased then decreased to reach 80%. The control positive group showed the highest SPA positive percent (100%) at the sixth week post-vaccination.

Table 2. SPA results of vaccinated and contact groups.

Group		Week Post Vaccination					
		1st	2nd	3rd	4th	5th	6th
1	F strain vaccinated group	8/10	10/10	10/10	10/10	10/10	10/10
2	Contact of F strain vaccinated group	6/10	10/10	10/10	10/10	10/10	8/10
3	ts-11 strain vaccinated group	10/10	10/10	10/10	10/10	10/10	8/10
4	Contact of ts-11 strain vaccinated group	6/10	6/10	6/10	10/10	10/10	8/10
5	6/85 strain vaccinated group	8/10	8/10	8/10	10/10	10/10	8/10
6	Contact of 6/85 strain vaccinated group	2/10	2/10	2/10	10/10	10/10	8/10
7	Control Positive	8/10	10/10	10/10	10/10	10/10	10/10
8	Control Negative	0/10	0/10	0/10	0/10	0/10	0/10

SPA= Serum plate agglutination

1.2. ELISA Results

Table 3 showed the ELISA results for the detection of *MG* antibodies in all groups. In F strain vaccinated group and its contact one, the geometric mean titre (GMT) was increasing from the first week (211.45 and 184.14, respectively) till reached 932.03 and818.81, respectively, at the end of the experiment. ts-11 and 6/85 strains vaccinated and contact groups, the GMT increased till reached 734.43, 703.04, 673.62 and 525.78, respectively, at the end of the experiment. The control positive group showed the highest GMT (1054.3) at the sixth week post-vaccination.

Table 3. GMT of ELISA results of serum collected from vaccinated and contact groups

	Group	Week Post Vaccination					
		1st	2nd	3rd	4th	5th	6th
1	F strain vaccinated group	211.45	360.19	856.61	873.49	898.3	932.03
2	Contact of F strain vaccinated group	184.14	223.3	366.25	431.29	705.88	818.81
3	ts-11 strain vaccinated group	211.98	320.82	435.18	492	676.14	734.43
4	Contact of ts-11 strain vaccinated group	138.21	188.38	337.12	343.23	644.26	703.04
5	6/85 strain vaccinated group	205.59	226.87	419.6	466.2	545.98	673.62
6	Contact of 6/85 strain vaccinated group	150.3	148.4	389.71	345.63	472.04	525.78
7	Control Positive	367.39	389.42	558.08	587.43	917.56	1054.3
8	Control Negative	48.92	65.23	16.39	83.72	7.59	20.87

GMT= Geometric mean titer of ELISA (-ve = 0: 148; ±ve = 149: 743; +ve: ≥ 744)

2. Isolation and PCR results of MG

Table 4 showed the results of isolation and PCR of different groups of chickens through the experiment. *MG* isolated from F strain vaccinated group increased and reached 100% at 2nd, 3rd, and 4th weeks post-vaccination then decreased at 5th, 6th weeks (isolation percent was 90%). Isolation percent from contact chickens of F strain vaccinated group increased from 50 % till 90% through the experiment. In ts-11 strain vaccinated group, *MG* isolation was increasing through the experiment till reached 80%, while its contact group reached 50%. In 6/85 strain vaccinated group and its contact, *MG* incidence was also increasing till reached 60% and 50%, respectively at the end of the experiment. *MG* isolation rate and PCR positive percent were 100% in the control positive group throughout the experiment. In F strain vaccinated group and its contact, PCR positive percent was increasing till reached 100% at the end of the experiment while in its contact group, it reached 90%. In ts-11 strain vaccinated group and its contact group, PCR positive percents reached 80% and 50%, respectively. While in 6/85 strain vaccinated group and its contact, it reached 60% and 50%, respectively.

Table 4. Isolation and PCR results of MG from all groups.

Group	Week post-vaccination											
	1st		2nd		3rd		4th		5th		6th	
	I*	P**	I*	P**	I*	P**	I*	P**	I*	P**	I*	P**
F strain vaccinated group	8/10	9/10	10/10	10/10	10/10	10/10	10/10	10/10	9/10	10/10	9/10	10/10
Contact of F strain vaccinated group	5/10	5/10	5/10	6/10	7/10	7/10	8/10	8/10	8/10	9/10	9/10	9/10
ts-11 strain vaccinated group	6/10	6/10	6/10	7/10	7/10	7/10	7/10	8/10	8/10	8/10	8/10	8/10
Contact of ts-11 strain vaccinated group	0/10	0/10	2/10	2/10	2/10	3/10	4/10	4/10	4/10	5/10	5/10	5/10
6/85 strain vaccinated group	3/10	3/10	4/10	4/10	4/10	4/10	5/10	5/10	6/10	6/10	6/10	6/10
Contact of 6/85 strain vaccinated group	0/10	0/10	0/10	0/10	0/10	1/10	2/10	2/10	5/10	5/10	5/10	5/10
Control Positive	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10

* = Isolation (positive isolates / no. examined by tracheal swabs)

**= PCR

3.Polymerase Chain Reaction Results

3.1.Results of PCR for *MG* from the F strain vaccinated chickens and its contact group

Photo (1) showed the *MG* specific band from vaccinated chickens by F strain vaccine and their contact group through six weeks during the experiment. All the examined isolates were identified as *MG* along the six weeks post vaccination (PV) of the experiment giving the specific band at 330bp.

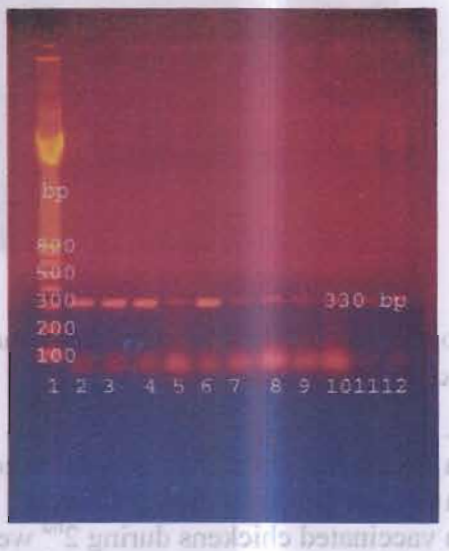

Photo 1. Agarose gel electrophoresis of PCR product from vaccinated chickens by F strain living vaccine and their contact

Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from F strain vaccinated chickens during 1st week.
Lane 3: *MG* from F strain contact chickens during 1st week.
Lane 4: *MG* from F strain vaccinated chickens during 2nd week.
Lane 5: *MG* from F strain contact chickens during 2nd week.
Lane 6: *MG* from F strain vaccinated chickens during 3rd week.
Lane 7: *MG* from F strain contact chickens during 3rd week.
Lane 8: *MG* from F strain vaccinated chickens during 4th week.
Lane 9: *MG* from F strain contact chickens during 4th week.
Lane 10: *MG* from F strain vaccinated chickens during 5th week.
Lane 11: *MG* from F strain contact chickens during 5th week.
Lane 12: *MG* from F strain vaccinated chickens during 6th week.

3.2.Results of Polymerase Chain Reaction for *MG* from the ts-11 strain vaccinated chickens and their contact group

Photo 2 showed the *MG* specific band obtained from vaccinated chickens by ts-11 strain vaccine and their contact group through six weeks during the experiment. Four isolates were identified as *MG* starting from the third to the sixth weeks PV in the ts-11 strain vaccinated group, while in the contact group two isolates were identified as *MG* in fifth and sixth weeks PV of the experiment giving the characteristic band at 330bp.

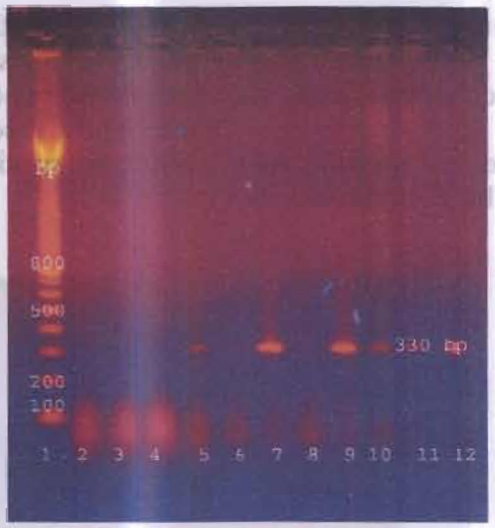

Photo 2. Agarose gel electrophoresis of PCR product from vaccinated chickens by ts-11 living *MG* vaccine and their contact

Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from ts-11 strain vaccinated chickens during 1st week.
Lane 3: *MG* from ts-11 strain contact chickens during 1st week.
Lane 4: *MG* from ts-11 strain vaccinated chickens during 2nd week.
Lane 5: *MG* from ts-11 strain vaccinated chickens during 3rd week.
Lane 6: *MG* from ts-11 strain contact chickens during 3rd week.
Lane 7: *MG* from ts-11 strain vaccinated chickens during 4th week.
Lane 8: *MG* from ts-11 strain contact chickens during 4th week.
Lane 9: *MG* from ts-11 strain vaccinated chickens during 5th week.
Lane 10: *MG* from ts-11 strain contact chickens during 5th week.
Lane 11: *MG* from ts-11 strain vaccinated chickens during 6th week.
Lane 12: *MG* from ts-11 strain contact chickens during 6th week.

3.3.Results of Polymerase Chain Reaction for *MG* from the 6/85 strain vaccinated chickens and their contact group

Photo 3 showed the *MG* specific band isolated from vaccinated chickens by 6/85 strain vaccine and their contact group through six weeks during the experiment. Four isolates were identified as *MG* starting from the third to the sixth weeks PV in the 6/85 strain vaccinated group. On the other hand, in the contact group isolates were positive from fifth week PV until the end of the experiment showing the characteristic band at 330bp.

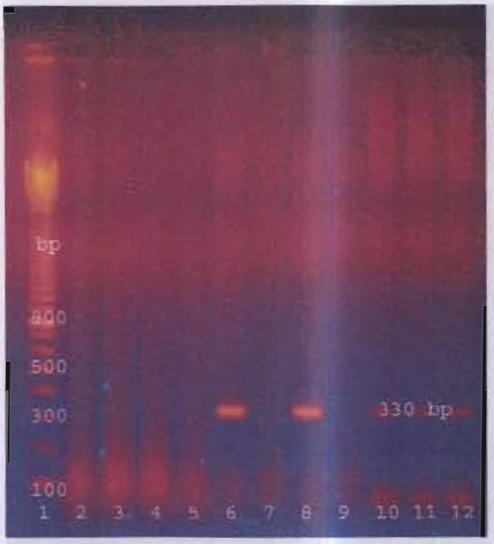

Photo 3. Agarose gel electrophoresis of PCR product from vaccinated chickens by 6/85 strain living *MG* vaccine and their contact.

Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from 6/85 strain vaccinated chickens during 1st week.
Lane 3: *MG* from 6/85 strain contact chickens during 1st week.
Lane 4: *MG* from 6/85 strain vaccinated chickens during 2nd week.
Lane 5: *MG* from 6/85 strain contact chickens during 2nd week.
Lane 6: *MG* from 6/85 strain vaccinated chickens during 3rd week.
Lane 7: *MG* from 6/85 strain contact chickens during 3rd week.
Lane 8: *MG* from 6/85 strain vaccinated chickens during 4th week.
Lane 9: *MG* from 6/85 strain contact chickens during 4th week.
Lane 10: *MG* from 6/85 strain vaccinated chickens during 5th week.
Lane 11: *MG* from 6/85 strain contact chickens during 5th week.
Lane 12: *MG* from 6/85 strain vaccinated chickens during 6th week.

4.Random Amplified Polymorphic DNA (RAPD) analysis Results

4.1.Results of Random Amplified Polymorphic DNA (RAPD) analysis for *MG* from the F strain vaccinated chickens

Random Amplified Polymorphic DNA (RAPD) was carried out for differentiation between *MG* virulent and vaccinal F strain. **Photo (4)** showed common bands at 383 and 510bp in all weeks PV except at second week, at 440bp in all weeks PV except at fifth one. Other common bands appeared at 361and 492bp in all weeks PV except at second and third and third and fourth weeks, respectively. RAPD analysis showed common bands at 410, 690 and 785bp in first and sixth weeks PV, at 900 and 1080bp in first, fifth and sixth weeks PV, at 810 and 980bp in second and third weeks PV, at 400bp in second, third and fourth weeks PV, at 601bp in first, third and sixth weeks PV, at 275bp in third and fifth weeks PV and at 300bp in fourth and fifth weeks PV.

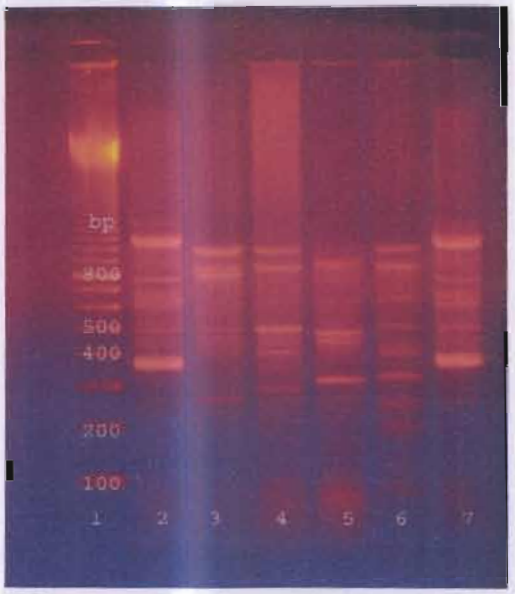

Photo 4. Electrophoretic analysis of RAPD patterns of *MG* from vaccinated chickens by F strain living vaccine.

Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from F strain vaccinated chickens during 1st week.
Lane 3: *MG* from F strain vaccinated chickens during 2nd week.
Lane 4: *MG* from F strain vaccinated chickens during 3rd week.
Lane 5: *MG* from F strain vaccinated chickens during 4th week.
Lane 6: *MG* from F strain vaccinated chickens during 5th week.
Lane 7: *MG* from F strain vaccinated chickens during 6th week.

4.2.Results of Random Amplified Polymorphic DNA (RAPD) analysis for *MG* from the contact group of F strain vaccinated chickens:

Photo 5 declared common bands at 383, 785, and 952bp in all weeks PV. Other common bands appeared at 300 and 638bp in all weeks PV except second and fourth weeks, at 275 and 410bp in fifth and sixth weeks PV. RAPD analysis also showed common bands at 440bp in all weeks PV except the fourth one and at 490bp in first, third and fourth weeks PV.

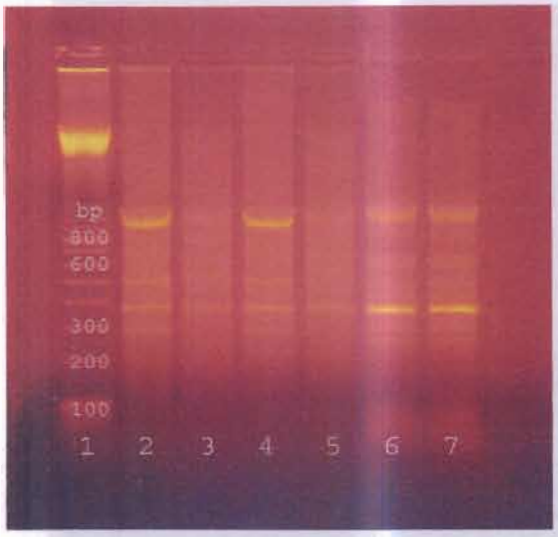

Photo 5. Electrophoretic analysis of RAPD patterns of *MG* from contact chickens with chickens vaccinated by F strain living vaccine.
Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from F strain contact chickens during 1st week.
Lane 3: *MG* from F strain contact chickens during 2nd week.
Lane 4: *MG* from F strain contact chickens during 3rd week.
Lane 5: *MG* from F strain contact chickens during 4th week.
Lane 6: *MG* from F strain contact chickens during 5th week.
Lane 7: *MG* from F strain contact chickens during 6th week.

4.3.Results of Random Amplified Polymorphic DNA (RAPD) analysis for *MG* from vaccinated chickens by ts-11 strain living *MG* vaccine and their contact group

Photo 6 showed common bands at 383 and 440bp in third, fourth, fifth and sixth weeks PV of the vaccinated group and in fifth and sixth weeks PV of the contact group. Other common bands appeared at 260, 510, 601, 650, 785, 1080, and 1025bp in third, fourth, fifth and sixth weeks PV of the vaccinated group and at 1070bp in fifth and sixth weeks PV of the contact group.

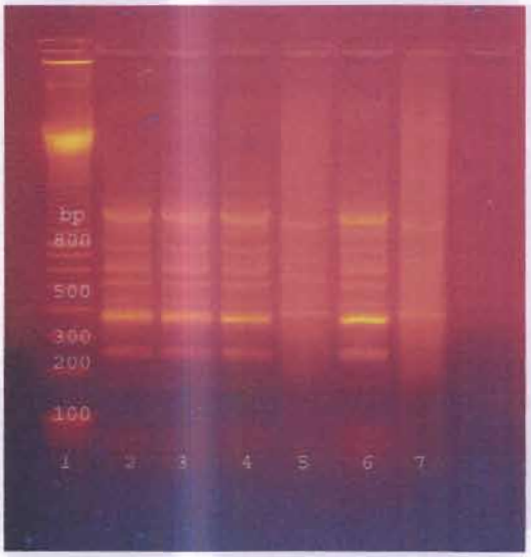

Photo 6. Electrophoretic analysis of RAPD patterns of *MG* from vaccinated chickens by ts-11 strain living vaccine and their contact.

Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from ts-11 strain vaccinated chickens during 3rd week.
Lane 3: *MG* from ts-11 strain vaccinated chickens during 4th week.
Lane 4: *MG* from ts-11 strain vaccinated chickens during 5th week.
Lane 5: *MG* from ts-11 strain contact chickens during 5th week.
Lane 6: *MG* from ts-11 strain vaccinated chickens during 6th week.
Lane 7: *MG* from ts-11 strain contact chickens during 6th week.

4.4.Results of Random Amplified Polymorphic DNA (RAPD) analysis for *MG* from vaccinated chickens by 6/85 strain living vaccine and their contact group

Photo 7 declared the presence of common bands at 383 and 440bp at third, fourth, and sixth weeks PV of the vaccinated group, and fifth and sixth weeks PV of the contact group. Other common bands appeared at 610bp in third, fourth, fifth and sixth weeks of the vaccinated group, and fifth and sixth weeks PV of the contact group. Also there were common bands appeared at 785, 980bp in fifth and sixth weeks PV of the vaccinated and contact groups. Other common bands appeared at 110,250, 300, 1050, and 1611bp in third and fourth weeks PV of the vaccinated chickens.

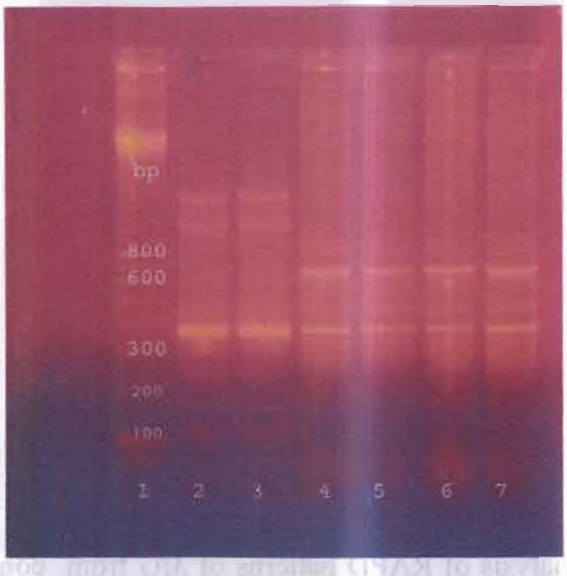

Photo 7. Electrophoretic analysis of RAPD patterns of *MG* from vaccinated chickens by 6/85 strain living vaccine and their contact.

Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from 6/85 strain vaccinated chickens during 3rd week.
Lane 3: *MG* from 6/85 strain vaccinated chickens during 4th week.
Lane 4: *MG* from 6/85 strain vaccinated chickens during 5th week.
Lane 5: *MG* from 6/85 strain contact chickens during 5th week.
Lane 6: *MG* from 6/85strain vaccinated chickens during 6th week.
Lane 7: *MG* from 6/85strain contact chickens during 6th week.

4.5.Results of Random Amplified Polymorphic DNA (RAPD) analysis for *MG* from control positive chickens

Common bands appeared at 383 and 1030bp in all weeks post vaccination. Other common bands appeared at 440, 528, 610, 785, 1050, 1210, and 1608bp in all weeks except the sixth one. These results were shown in Photo 8.

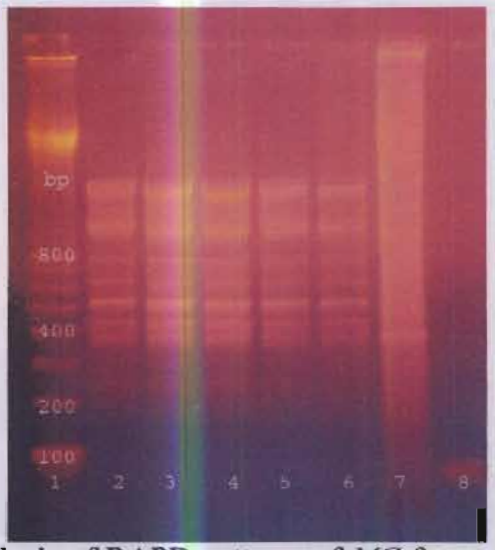

Photo 8. Electrophoretic analysis of RAPD patterns of *MG* from control positive and negative chickens.

Lane 1: 100 bp DNA Ladder.
Lane 2: *MG* from control positive chickens during 1st week.
Lane 3: *MG* from control positive chickens during 2nd week.
Lane 4: *MG* from control positive chickens during 3rd week.
Lane 5: *MG* from control positive chickens during 4th week.
Lane 6: *MG* from control positive chickens during 5th week.
Lane 7: *MG* from control positive chickens during 6th week.
Lane 8: Control negative.

Table 5 showed common DNA bands of *MG* from the vaccinated groups and the control positive one. Three common DNA bands of *MG* appeared at 383, 440 and 785bp among the vaccinated groups (F, ts-11 and 6/85) and the control positive one (virulent *MG* field isolate from broiler breeder flocks and identification was confirmed by PCR, RAPD and sequencing of 16S gene). One common DNA band of *MG* was detected at 610bp between *MG* from the 6/85 vaccinated group and the control positive one (challenge *MG* strain). So *MG* DNA bands from the 6/85 vaccinated group was more similar to that of the challenge *MG* virulent strain.

Table 5. Common DNA bands of *MG* from the vaccinated groups and the control positive one.

Vaccinated groups	F- Strain	ts-11 Strain	6/85 Strain	Control Positive
Common DNA bands	383	383	383	383
	440	440	440	440
	785	785	785	785
			610	610

DISCUSSION

None of the vaccines afforded complete protection against infection, but some was effective in suppression the multiplication of the organism, resulting in less tissue damage followed by faster recovery*(12,13)*. Live *MG* vaccines include the F strain and attenuated

strains ts-11 and 6/85. F strain reduces the decline in egg production and has been used to displace endemic strains in multi-age flocks. The major disadvantage is the inherited virulence of F strain. Strain ts-11 is less virulent, less infectious than F strain. It provides somewhat weaker, but usually effective long term protective immunity, which is vaccine-dose dependent. This strain stimulates a detectable although variable systemic antibody response. Strain 6/85 also stimulates a weaker protective immune response than F strain and appears not to persist in vaccinated birds beside; it may fail to stimulate a detectable systemic antibody response *(14)*.

Serum plate agglutination (SPA) test and Enzyme-linked immunosorbent assay (ELISA) were used for evaluation of the immune response in the vaccinated groups. SPA test could detect positive *MG* antibodies in vaccinated and contact groups in which the positive percent was 100% in F strain vaccinated group and the control positive one at the end of the experiment, but it was 80% in ts-11, 6/85 vaccinated and contact groups and in the contact group of F strain vaccinated chickens. As for ELISA test, the strongest serological response against *MG* could be detected in control positive group in the sixth week PV (1054.32), followed by F strain vaccinated and contact groups, then ts-11 vaccinated and contact groups (932.03, 818.81, 734.43 and 703.04, respectively) and the weakest serological response was detected in 6/85 vaccinated and contact groups (673.62 and 525.78, respectively) at the end of the experiment (Tables 3, 4).

Positive response was detected in 90% of ts-11 vaccinated birds at 42 day PV by SPA *(15)*. *MG* ELISA detected positive immune response in ts-11 vaccinated birds (10-70%) at 42, 63, 84 and 105 days PV and commingled pullets (10%) at 42 and 105 days PV. However, on contrary it has been recorded that the 6/85 strain vaccinated birds where no positive *MG* antibodies could be detected by SPA *(6)*. Also 6/85 vaccinated chickens may exhibit a poor to negative serologic response *(6)*. The F strain vaccine produced superior sero-conversion and good protection than ts-11 and 6/85 strain vaccines *(16)*.

In the present study, polymerase chain reaction (PCR) was used for the detection of *MG* in different groups of the experiment. In F strain vaccinated group, 90-100% were positive (identified as *MG* giving the specific DNA fragment at 330bp) till the end of the experiment. The use of F strain vaccine induced protection against infection with field strains of *MG*, while in its contact group 50-90% were positive *(17)*.

Concerning ts-11 strain vaccinated group, 60-80% were positive (identified as *MG* giving the characteristic DNA fragment at 330bp) for *MG*-PCR and 0-50% were positive in its contact group. As for 6/85 strain vaccinated group, 30-60% were positive and 0-50% were positive in its contact group. On the other hand, the control positive group was 100% for *MG*-PCR until the end of the experiment, but the control negative group was negative till the end of the experiment.

The transmissibility of live *MG* vaccine strains ts-11 and 6/85 from vaccinated layer pullets to commingled birds shoed that strain ts-11 was recovered from 60-90% of vaccinated birds and 0-40% of commingled pullets. Strain 6/85 was recovered from 0-20% of vaccinated birds, but not from commingled pullets.

In the present work, random amplified polymorphic DNA (RAPD) was used for characterization of *MG* isolates from F strain vaccinated group. Four common DNA bands could be detected in all the isolated strains during the experiment, these bands were at 383, 440, 490, and 510bp, while in the F strain vaccinated group, the isolated strains shared three common DNA bands at 383, 440, and 785bp. The isolates were considered identical when major bands were similar *(18)*.

Concerning ts-11 strain vaccinated group, DNA banding patterns of all the isolated strains during the experiment were identical, having nine similar DNA bands (ranged from 260 to 1250bp). Also two strains

isolated from ts-11 contact group had two common DNA bands at 383 and 410bp in the fifth and sixth weeks PV, which were detected in the isolates of the vaccinated group. *MG* was isolated from ts-11 vaccinated flocks up to 100 weeks of age, all such isolates tested by RAPD were ts-11 type ***(19)***. These results indicated a potential use of ts-11 in *MG* eradication program.

As for 6/85 strain vaccinated group and its contact one, *MG* strain isolated from the vaccinated group at the third and fourth week PV were identical, the number of DNA bands was eight ranged from 110 to 1611bp. However, *MG* strains isolated from vaccinated and contact groups at the fifth and sixth weeks PV had different DNA patterns when compared with those isolated from vaccinated group at the third and fourth weeks PV.

RAPD technique was used to differentiate between *MG* virulent strain used in the control positive group and the vaccinal strains. *MG* strain isolated from the control positive chickens exhibited identical DNA banding pattern, including nine DNA bands ranging from 383 to 1608bp from the first to the fifth week PV, while the DNA profile at the sixth week had only two bands at 383 and 1030bp. The isolates are considered identical when major band differences could not be visualized ***(18)***.

MG strain common DNA bands from the 6/85 vaccinated group was more similar to those of the challenge *MG* virulent field isolate.

REFERENCES

1.Jordan, F T W (1979). Avian *Mycoplasmas*. In J.G Thully and R.F. White comb (ed.). Academic press Inc. New York, N.Y.The *mycoplasmas*. II: 1-48.

2.Soeripto, M (1987). Pathogenicity and immunogenicity of *MG*, Ph.D. Thesis, The University of Melbourne.

3.Whithear, K G ; Soeripto; Harrigan, K E ; and Ghiocas; E (1990). Immunogenicity of a temperature sensitive mutant *MG* vaccine, 67: 168-174.

4.Evans, R D and Hafez, Y S (1992). Evaluation of *MG* strain exhibiting reduced virulence for prevention and control of poultry *mycoplasmosis*. Avian Dis., 36: 197-201.

5.Branton, S L ; Bearson, M D ; Bearson, B ; Lott, B D ; Maslin, W R ; Collier, S D ; Pharr, G T and BoyKin, D L (2002). The effects of 6/85 live *MG* vaccine in commercial layer hens over 43 week laying cycle on egg production, selected egg quality parameters, and egg size distribution when challenged before beginning of lay. Avian Dis., 46: 423-428.

6.Thorne Steinlage, S J ; Ferguson, N ; Sander, J E ; Garcia, M ; Subramanian, S ; Leiting, V A and Kleven, S H (2003). Isolation and characterization of a 6/85-like *MG* from commercial laying hens. Avian Dis., 47: 499-505.

7.Frey, M C ; Hanson, R P and Anderson, D P (1968). A medium for the isolation of avian Mycoplasma. Ann. Vet. Res., 29: 2164-2171.

8.Lin, M Y and Kleven, S H (1984). Evaluation of attenuated strains of *MG* as vaccines in young chickens. Avian Dis., 28: 88-99.

9.Higgins, P A and Whithear, K G (1986). Detection and differentiation of *MG* and *M. Synoviae* antibodies in chicken serum. Avian Dis., 30, (1):160-168.

10.Kempf, I ; Blanchard, A ; Gesbert, F ; Guittet, M and Bennejean, G (1993). The polymerase chain reaction for *MG* detection. Avian Pathol., 22 : 739-750.

11.Fan, H H ; Kleven, S H and Jackwood, M W (1995). Application of polymerase chain reaction with arbitrary primers to strain identification of *MG*. Avian Dis., 39: 729-735.

12.Hildebrand, D G ; Page, D E and Berg, J R (1983). *MG* laboratory and field studies evaluating the safety and efficacy of an inactivated *MG* bacterin. Avian Dis., 27: 792-802.

13.Rodriguiz, R and Kleven, S H (1985). Evaluation of a vaccine against *MG* in commercial in broilers. Avian Dis., 24: 879-889.

14.Whithear, K G (1996). Control of avian *mycoplasmosis* by vaccination. Rev. Sci. Tech. Off. Int. Epiz.,15: 1527-1553.

15.Ley, D H ; Mclaren, J M ; Miles, A M ; Barnes, H J ; Miller, S H and Franz, G (1997). Transmissibility of live *MG* vaccine strains ts-11 and 6/85 from vaccinated layer pullets to sentinel poultry. Avian Dis., 41, (1): 187-194.

16.Seif El-din, M ; Aly, M and Moussa, S (2000). Field evaluation of *MG* vaccines in broiler breeder chickens. 9th Sci. Con., Fac. Vet. Med. Assuit Univ. Egypt.

17.Cummings, T S and Kleven, S H (1986). Evaluation of protection against *MG* infection in chickens vaccinated with F strain of *MG*. Avian Dis.; 30: 169-171.

18.Ferguson, N M ; Hepp, D ; Sun, S ; Lkuta N ; Levisohn S ; Kleven, S H and Garcia, M (2005). Use of molecular diversity of *MG* by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. Microbiology, 151: 1883-1893.

19.Turner, K S and Kleven, S H (1998). Eradication of live F strain *MG* vaccine using live ts-11 on multiage commercial farms. Avian Dis., 42: 404-407.

الملخص العربى

تحضير لقاحات حية جديدة للميكوبلازما جاليسيبتكم وتقييمها فى الدواجن

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هذه الدراسة تضمنت تحضير لقاحات حية جديدة للميكوبلازما جاليسبتيكم (ts-11، 6/85) و تقييمها مع عترة F فى الدجاج من خلال تجربة معملية ، مقارنة تأثير هذه اللقحات فى الدجاج ، وإستخدام إختبارى سلسلة تفاعل إنزيم البوليمريز وتفاعل إنزيم البوليمريز العشوائى للكشف عن عترات اللقاح.

تضمنت التجربة عدد ٨٠ كتكوت عمر يوم خالى من الميكوبلازما مقسمة إلى ثمانية مجموعات. تم تحصين المجموعات عند عمر ٢٨ يوم عن طريق التقطير فى الأنف بجرعة ٠,١ مل. المجموعة الأولى تم تحصينها بلقاح عترة F و المجموعة الثانية كانت مجاورة لها. المجموعة الثالثة تم تحصينها بلقاح عترة ts-11 و المجموعة الرابعة كانت مجاورة لها. المجموعة الخامسة تم تحصينها بلقاح عترة 6/85 و المجموعة السادسة كانت مجاورة لها. أما المجموعتين السابعة و الثامنة فكانتا الضابط الإيجابى و السلبى للتجربة.تم تجميع عينات دم و مسحات من القصبة الهوائية من الأسبوع الأول حتى السادس بعد التحصين.

إستطاع إختبار التلازن الدموى أن يكشف عن الأجسام المناعية للميكوبلازما جاليسبتيكم بنسبة ١٠٠% فى المجموعة الأولى والسابعة. أما بالنسبة للمجموعات الأخرى فقد إستطاع إختبار التلازن الدموى الكشف عن ٨٠% من الأجسام المناعية للميكوبلازما جاليسبتيكم ماعدا المجموعة الثامنة.كما تم إستخدام

إختبار الإليزا فى تقييم المستوى المناعى للدجاج. فقد كان أعلى متوسط مستوى للأجسام المناعية فى المجموعة السابعة (١٠٥٤,٣٢) ويليها المجموعة الثانية والثالثة (٩٣٢,٠٣، ٨١٨,٨١) بالترتيب. أما بالنسبة للمجموعات الثالثة، الرابعة، الخامسة، والسادسة فكان متوسط مستوى الأجسام المناعية (٧٣٤,٤٣، ٧٠٣,٠٤، ٦٧٣,٦٢، ٥٢٥,٧٨) بالترتيب.

تم عزل الميكوبلازما جاليسبتيكم بنسبة ١٠٠% من مجموعتى الأولى والسابعة. أما فى المجموعة الثانية فقد كانت نسبة العزل تزداد من ٥٠-٩٠%. إزدادت نسبة عزل الميكوبلازما جاليسبتيكم فى المجموعة الثالثة لتصل إلى ٨٠% ، ولكن فى المجموعة الرابعة وصلت نسبة العزل إلى ٥٠% فقط . إزدادت نسبة عزل الميكوبلازما جاليسبتيكم فى المجموعة الخامسة والسادسة حتى وصلت إلى ٥٠% و ٦٠% بالترتيب.

أثبت إختبار سلسلة تفاعل إنزيم البوليمريز وجود حزمة من الحامض النووى مميزة للميكوبلازما جاليسبتيكم عند ٣٣٠ زوج من القواعد فى المجموعة الأولى بنسبة (٩٠ -١٠٠%) وفى المجموعة الثانية بنسبة (٥٠ - ٩٠%) ،وفى المجموعة السابعة بنسبة (١٠٠%). وقد إختلفت هذه النسبة فى المجموعات الأخرى لتصل إلى (٦٠ - ٨٠%)فى المجموعة الثالثة ،(٠ - ٥٠%) فى المجموعة الرابعة، و(٣٠ -٦٠%) ، (٠ – ٥٠%) فى المجموعةالخامسة والسادسة بالترتيب.

وقد إكد إختبار تفاعل إنزيم البوليمريز العشوائى وجود عترة F فى المجموعة الأولى والثانية. وبالتالى فإن لقاح عترة F إستطاع مقاومة العترة الضارية المستخدمة فى إختبار التحدى. أما بالنسبة للمجموعة الثالثة فقد إستطاع إختبار تفاعل إنزيم البوليمريز العشوائى الكشف عن عترة ts-11 فى الأسبوع الثالث بعد التحصين و حتى نهاية التجربة. ولكن فى المجموعة الرابعة ظهرت هذه العترة فى الأسبوع الخامس والسادس بعد التحصين. نستنتج من ذلك أن لقاح عترة ts-11 أعطى حماية للكتاكيت أقل من لقاح عترة F. هذا الإختبار أوضح وجود عترة 6/85 فى الأسبوع الثالث والرابع بعد التحصين فى المجموعة الخامسة. وعلى الجانب الآخر فإن الميكوبلازما جاليسبتيكم المعزولة من هذه المجموعة أظهرت إختلاف فى الحامض النووى لعترة 6/85 ، ولكن كانت متشابهه فى الأسبوع الخامس والسادس مع العترة الضارية المستخدمة فى إختبار التحدى. نستنتج من ذلك أن لقاح عترة 6/85 لم يستطع مقاومة العترة الضارية المستخدمة فى إختبار التحدى.