OPEN ACCESS
Journal of Animal Health and Production
Research Article

# Efficacy Assessment of Avian *Pasteurella multocida* and *Mycoplasma gallisepticum* Local Vaccines

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**Abstract** | Diseases caused by *Pasteurella multocida* (*P. multocida*) and *Mycoplasma gallisepticum* (*M. gallisepticum*) are of significant importance and induce great losses in poultry industry, so vaccination against diseases caused by both organisms is crucial. The present study was conducted to prepare inactivated vaccines from local strains of *P. multocida* and *M. gallisepticum* either alone or in combinations, evaluate these vaccines through measuring the immune response as well as detect the protection rates against the challenge with virulent *P. multocida* and *M. gallisepticum* strains. The prepared vaccine was evaluated by determination of the cellular immunity by heterophils/lymphocytes ratio (H/L) and evaluation of the humoral immunity by indirect haemagglutination (IHA) and haemagglutination inhibition (HI) tests. The potency of the vaccine was evaluated by the passive mouse protection and challenge tests against the challenge with the virulent strains of *P. multocida* (serotypes A and D) and *M. gallisepticum* (Eis3-10 strain). The results revealed that the combined inactivated *P. multocida* and *M. gallisepticum* vaccine adjuvanted with Montanide ISA70 induced high and long duration of antibody response and significant protection against the challenge with virulent strains of *P. multocida* and *M. gallisepticum*. In conclusion, the locally prepared combined inactivated *P. multocida* and *M. gallisepticum* vaccine elicited good cellular and humoral immune responses as well as high protection of chickens against both diseases.

**Keywords** | Fowl cholera (FC), Haemagglutination inhibition (HI), Heterophils/lymphocytes (H/L), *Mycoplasma gallisepticum* (*M. gallisepticum*), *Pasteurella multocida* (*P. multocida*).

**Received** | January 12, 2021; **Accepted** | May 09, 2021; **Published** | June 25, 2021
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**Citation** | Ibrahim FF, Abd El-Ghany WA, El-Rawy EM, Shaker MM, El-Jakee J (2021). Efficacy assessment of avian *pasteurella multocida* and *mycoplasma gallisepticum* local vaccines. J. Anim. Health Prod. 9(3): 213-221.
**DOI** | http://dx.doi.org/10.17582/journal.jahp/2021/9.3.213.221
**ISSN** | 2308-2801



## INTRODUCTION

Fowl cholera (FC) is a highly contagious disease caused by *Pasteurella multocida* (*P. multocida*) and has been recognized as an important disease in poultry for more than 20 years (Furian et al., 2016). The disease causes devastating economic losses in poultry industry through death, weight loss and condemnation of carcasses (Xiao et al., 2015). Vaccination against FC is considered as one of the most world-wide preventive measures to reduce the prevalence of the disease condition (Kardos and Kiss, 2005; Parvin et al., 2011). Inactivated *P. multocida* vaccines are widely used with successful results (Akhtar et al., 2016; Salama et al., 2019). Local vaccines of *P. multocida* that prepared mainly from inactivated whole bacterial cells induced protective immune response and good protection of birds against challenge (Glisson et al., 2008; Qandoos, 2018).

*Mycoplasma gallisepticum* (*M. gallisepticum*) infection is a complex, complicating and multifactorial disease posing a serious economic challenge to the prosperity of poultry enterprise in many parts of the world. *M. gallisepticum* is con-

sidered as an economically important respiratory disease problem for commercial and backyard poultry production systems (Talha, 2003). Infection with *M. gallisepticum* could resulting in high morbidity, poor feed conversion, decreased production, medication costs and high mortality when complicated with other infections (Mallinath and Hari Babu, 2013). The sero-prevalence of *M. gallisepticum* antibodies in broiler breeder flocks was 52.92% using Enzyme linked immuno sorbent assay (EI-Jakee et al., 2019). Prevention and control programs of avian mycoplasmosis are based on strict biosecurity, surveillance and eradication of infected breeder flocks (Raviv et al., 2008). Vaccination against *M. gallisepticum* can be a useful long term solution in situation where maintaining flocks free of infection is not feasible, especially in multiage commercial egg production sites (Kleven, 2008; Jacob et al., 2014).

Although development of different types of live and inactivated vaccines against FC and mycoplasmosis, both diseases are still circulating in commercial poultry farms and this situation necessitates the development of local vaccines from the predominant circulating field strains.

So, this study was planned to prepare inactivated vaccines from local strains of *P. multocida* and *M. gallisepticum* either alone or in combinations, evaluate these vaccines through measuring the immune response as well as detect the protection rates against the challenge with virulent *P. multocida* and *M. gallisepticum* strains.

## MATERIALS AND METHODS

### Preparation of Local Bacterins

Inactivated oil emulsion *P. multocida* and *M. gallisepticum* bacterins were prepared as Mukkur et al. (1982) and Yoder (1979); respectively. Equal parts of *P. multocida* serotypes (A and D) (Serotypes A and D were kindly obtained from Aerobic Bacterial Vaccines Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo) and *M. gallisepticum* (Field isolate of *M. gallisepticum* (Eis3-10) was kindly obtained from Mycoplasma Department, Animal Health Research Institute, Dokki, Giza, Egypt) were mixed. Equal amount of above culture was thoroughly mixed with Montanide ISA70 oil adjuvant and finally thiomersal was added at a final concentration of 0.01%. The prepared bacterin was tested for sterility from any other bacterial or fungal contaminants, purity and safety after inoculation in chickens and mice.

### Experimental Design

A total of 135, 4 weeks old specific pathogen free chickens were divided into 4 groups. The 1st group was 30 birds and vaccinated with *P. multocida* bacterin (G1), the 2nd group was 15 birds and vaccinated with *M. gallisepticum* bacterin (G2), the 3rd was 45 birds vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin (G3) and the 4th group was 45 birds and kept as control non vaccinated chickens (G4). Each chickens in the vaccinated group received 0.5 ml of the tested vaccines subcutaneously (S/C) in a double doses with one month interval. The 1st and 2nd vaccine doses were at 4 and 8 weeks old; respectively. The study was done in accordance with the National Regulations on Animal Welfare and Institutional Animal Ethical Committee Recommendations and Approval.

### Determination of Cellular Immunity

Relative proportion of heterophils to lymphocytes (H/L ratio) was determined in the staining blood films collected from chickens groups (Cotter, 2015). Blood samples were collected at the 3rd, 7th and 15th days after the first and second vaccinations and after the challenge. Blood films were examined to obtain counts of lymphocytes and granulocytes per 100 leukocytes. Obtained cell counts were used for calculation of H/L ratio.

### Evaluation of Humoral Immunity

Serum samples were collected every 2 weeks till 25 weeks old for determination of antibody titers in vaccinated *P. multocida* types A and D (Sawada et al., 1982) and *M. gallisepticum* (Senterifit, 1983) chickens groups using indirect haemagglutination (IHA) and haemagglutination inhibition (HI) tests.

### Evaluation of Bacterin Potency

Passive mouse protection test was done to evaluate the protection rate of sera collected from chickens groups vaccinated with either *P. multocida* types (A and D) or combined *P. multocida* and *M. gallisepticum* bacterins (Tabatabaei et al., 2007). About 0.2 ml of the sera of vaccinated above mentioned groups was S/C inoculated in 120 mice while 60 mice were kept as control. After 24 hours, each of vaccinated mice was challenged separately and S/C with 0.1 ml virulent *P. multocida* types (A and D) containing 100 lethal dose 50 ($LD_{50}$). As well, challenge test for 11 weeks old chickens (3 weeks post-2nd vaccination) was done as OIE (2012), where each chicken in vaccinated groups was challenged with the same route and dose as mice. Both mice and chickens were kept under observation for 7 days later.

### Statistical Analysis

Paired t-test was used for comparison of H/L ratio, antibody titers between G1 and G3 and between G2 and G3. ANOVA test was conducted for comparison of H/L ratio, antibody titers between vaccinated groups and control group. The level of significance for all statistical tests was set at ($p \leq 0.05$). All statistical tests were performed through the statistical package for social studies (SPSS) version 19 for windows (IBM SPSS, Chicago, IL, USA).

**Table 1:** Evaluation of H/L ratio post vaccination with different vaccines in chickens

| Interval times of blood collection | Groups | | | |
|---|---|---|---|---|
| | G1 | G2 | G3 | G4 |
| Pre-vaccination | 1.3 | 1.4 | 1.1 | 1.5 |
| 1st vaccination | | | | |
| At 3rd day | 0.7 | 0.8 | 0.5 | 1.4 |
| At 7th day | 0.4 | 0.6 | 0.2 | 1.0 |
| At 15th day | 0.5 | 0.7 | 0.3 | 1.3 |
| Booster vaccination | | | | |
| At 3rd day | 0.3 | 0.5 | 0.2 | 1.2 |
| At 7th day | 0.1 | 0.4 | 0.1 | 0.9 |
| At 15th day | 0.2 | 0.5 | 0.2 | 1.3 |
| Challenge | | | | |
| At 3rd day | 0.2 | 0.4 | 0.2 | 1.2 |
| At 7th day | 0.1 | 0.3 | 0.1 | 1.0 |
| At 15th day | 0.4 | 0.6 | 0.3 | 1.5 |

G1= *P. multocida* bacterin, G2= *M. gallisepticum* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control
1st vaccination at 4 weeks old, Booster vaccination at 8 weeks old, Challenge at 11 weeks old

**Table 2:** The level of antibody titers against *P. multocida* type (A) in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin using IHA

| Interval time of serum collection | Groups | | |
|---|---|---|---|
| | G1 | G3 | G4 |
| Pre-vaccination | 2 | 2 | 0 |
| 1st vaccination | | | |
| 2 weeks post 1st vaccination | 64 | 128 | 2 |
| Booster vaccination | | | |
| 2 weeks post 2nd vaccination | 256 | 256 | 2 |
| Challenge | | | |
| 2 weeks post-challenge | 128 | 128 | 4 |
| 4 weeks post-challenge | 128 | 512 | 2 |
| 6 weeks post-challenge | 512 | 1024 | 2 |
| 8 weeks post-challenge | 256 | 512 | 0 |
| 10 weeks post-challenge | 256 | 512 | 0 |
| 12 weeks post-challenge | 256 | 256 | 0 |
| 14 weeks post-challenge | 128 | 128 | 0 |

G1= *P. multocida* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control
1st vaccination at 4 weeks old, Booster vaccination at 8 weeks old, Challenge at 11 weeks old

**Table 3:** The level of antibody titers against *P. multocida* type (D) in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin using IHA

| Interval time of serum collection | Groups | | |
|---|---|---|---|
| | G1 | G3 | G4 |
| Pre-vaccination | 2 | 2 | 0 |
| 1st vaccination | | | |
| 2 weeks post 1st vaccination | 32 | 64 | 2 |
| Booster vaccination | | | |
| 2 weeks post 2nd vaccination | 64 | 128 | 2 |

| | | | |
|---|---|---|---|
| Challenge | | | |
| 2 weeks post-challenge | 128 | 128 | 2 |
| 4 weeks post-challenge | 256 | 512 | 4 |
| 6 weeks post-challenge | 512 | 512 | 2 |
| 8 weeks post-challenge | 128 | 256 | 2 |
| 10 weeks post-challenge | 64 | 64 | 0 |
| 12 weeks post-challenge | 64 | 64 | 0 |
| 14 weeks post-challenge | 32 | 32 | 0 |

G1= *P. multocida* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control
1st vaccination at 4 weeks old, Booster vaccination at 8 weeks old, Challenge at 11 weeks old

**Table 4:** The level of antibody titers against *M. gallisepticum* in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin using HI

| **Interval time of serum collection** | **Groups** | | |
|---|---|---|---|
| | **G2** | **G3** | **G4** |
| Pre-vaccination | 2 | 2 | 0 |
| 1st vaccination | | | |
| 2 weeks post 1st vaccination | 32 | 64 | 2 |
| Booster vaccination | | | |
| 2 weeks post 2nd vaccination | 64 | 128 | 2 |
| Challenge | | | |
| 2 weeks post-challenge | 128 | 256 | 4 |
| 4 weeks post-challenge | 128 | 512 | 2 |
| 6 weeks post-challenge | 128 | 512 | 2 |
| 8 weeks post-challenge | 64 | 256 | 0 |
| 10 weeks post-challenge | 64 | 128 | 0 |
| 12 weeks post-challenge | 32 | 64 | 0 |
| 14 weeks post-challenge | 16 | 64 | 0 |

G2= *M. gallisepticum* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control
1st vaccination at 4 weeks old, Booster vaccination at 8 weeks old Challenge at 11 weeks old

**Table 5:** Passive mouth protection test against challenge with *P. multocida* type (A) in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin

| **Interval times of serum collection** | **Total number of mice** | **Groups** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **G1** | | | **G3** | | | **G4** | | |
| | | **D** | **S** | **P%** | **D** | **S** | **P%** | **D** | **S** | **P%** |
| Pre-vaccination | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 |
| 1st vaccination | | | | | | | | | | |
| 2 weeks post 1st vaccination | 5 | 1 | 4 | 80 | 0 | 5 | 100 | 5 | 0 | 0 |
| Booster vaccination | | | | | | | | | | |
| 2 weeks post 2nd vaccination | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| Challenge | | | | | | | | | | |
| 2 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| 4 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| 6 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| 8 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |

P%= Number of survived mice/Total number of mice X 100
S= Survived mice, D= Dead mice, G1= *P. multocida* bacterin, G3= Combined *P. multocida*and *M. gallisepticum* bacterin
G4= Control 1st vaccination at 4 weeks old, Booster vaccination at 8 weeks old, Challenge at 11 weeks old

**Table 6:** Passive mouth protection test against challenge with *P. multocida* type (D) in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin

| Interval times of serum collection | Total number of mice | Groups | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G1 | | | G3 | | | G4 | | |
| | | D | S | P% | D | S | P% | D | S | P% |
| Pre-vaccination | 5 | 5 | 0 | 0 | 5 | 0 | 0 | 5 | 0 | 0 |
| 1st vaccination | | | | | | | | | | |
| 2 weeks post 1st vaccination | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| Booster vaccination | | | | | | | | | | |
| 2 weeks post 2nd vaccination | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| Challenge | | | | | | | | | | |
| 2 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| 4 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| 6 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |
| 8 weeks post challenge | 5 | 0 | 5 | 100 | 0 | 5 | 100 | 5 | 0 | 0 |

P%= Number of survived mice/Total number of mice X 100; S= Survived mice; D= Dead mice
G1= *P. multocida* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control
1st vaccination at 4 weeks old, Booster vaccination at 8 weeks old, Challenge at 11 weeks old

**Table 7:** Challenge test against *P. multocida* type (A) in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin

| Groups | G1 | G3 | G4 |
|---|---|---|---|
| Total number of chickens | 15 | 15 | 15 |
| D | 1 | 0 | 15 |
| S | 14 | 15 | 0 |
| P% | 93 | 100 | 0 |

P%= Number of survived chickens/Total number of chickens X 100
S= Survived chickens, D= Dead chickens
G1= *P. multocida* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control

**Table 8:** Challenge test against *P. multocida* type (D) in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin

| Groups | G1 | G3 | G4 |
|---|---|---|---|
| Total number of chickens | 15 | 15 | 15 |
| D | 0 | 0 | 15 |
| S | 15 | 15 | 0 |
| P% | 100 | 100 | 0 |

P%= Number of survived chickens/Total number of mice X 100
S= Survived chickens, D= Dead chickens
G1= *P. multocida* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control

**Table 9:** Challenge test against *M. gallisepticum* in chickens vaccinated with combined *P. multocida* and *M. gallisepticum* bacterin

| Groups | G2 | G3 | G4 |
|---|---|---|---|
| Total number of chickens | 15 | 15 | 15 |
| Birds with respiratory manifestations | 3 | 1 | 15 |
| P% | 80 | 93 | 0 |

P%= Number of survived chickens/Total number of chickens X 100
G2= *M. gallisepticum* bacterin, G3= Combined *P. multocida* and *M. gallisepticum* bacterin, G4= Control

## RESULTS

The prepared vaccines were free from any bacterial and fungal contaminations. They were pure and proved their safety after inoculation in chickens and mice without signs or mortalities.

The data illustrated in Table (1) reveals that the H/L ratio at 7th day post 1st vaccination for G1, G2 and G3 was 0.4, 0.6 and 0.2; respectively in comparison with 1.0 in G4. However, at 7th day post 2nd vaccination, the ratio for G1, G2 and G3 was 0.1, 0.4 and 0.1; respectively but 0.9 in G4. The H/L ratio for G1, G2 and G3 at the 7th day after challenge was 0.1, 0.3 and 0.1; respectively while 1.0 in G4. Using ANOVA, there were significant differences ($p \leq 0.05$) in H/L ratio between vaccinated groups (G1, G2 and G3) and the control one (G4).

From the results of IHA in Table (2), it can noticed that the antibody titers against *P. multocida* type (A) at 2 weeks post 1st vaccination were 64 and 128 for G1 and G3; respectively compared with 2.0 in G4. The antibody titers at 2 weeks post 2nd vaccination were 256 for both G1 and G3 in comparison with 2.0 in G4. At 6 weeks post-challenge, the titers were 512 and 1024 for G1 and G3 while 2.0 for G4.

Table (3) shows that the IHA antibody titers against *P. multocida* type (D) at 2 weeks post 1st vaccination were 32 and 64 for G1 and G3; respectively but 2.0 for G4. The titer was 64 for G1, 128 for G3 and 2.0 for G4 at 2 weeks post 2nd vaccination. The antibody titers at 6 weeks post challenge were 512 for G1 and G3 and 2.0 for G4.

It was observed significant differences ($p \leq 0.05$) in antibody titers against *P. multocida* type (A) and type (D) between vaccinated groups (G1 and G3) and the control one (G4).

The data demonstrated in Table (4) reveals that the levels of HI antibody titers against *M. gallisepticum* at 2 weeks post 1st vaccination were 32 for G2, 64 for G3 and 2.0 for G4. But at 2 weeks post 2nd vaccination, the titers were 46 and 128 for G2 and G3; respectively compared with 2.0 for G4. The HI titers at 6 weeks post challenge for G2, G3 and G4 were 128, 512 and 2.0; respectively.

There were significant differences ($p \leq 0.05$) in antibody titers against *M. gallisepticum* between vaccinated groups (G2 and G3) and the control one (G4).

As shown in Table (5), the protection percentage (P%) against challenge of mice with virulent strain of *P. multocida* type (A) at 2 weeks post 1st vaccination was 80 and 100% for G1 and G3; respectively compared with 0% in G4. At 2 weeks post 2nd vaccination and 8 weeks post challenge, the P% were 100% for both G1 and G3 while 0% in G4.

The results in Table (6) shows that the P% against challenge of mice with virulent strain of *P. multocida* type (D) at 2 weeks post 1st vaccination was 100% for both G1 and G3 but 0% for G4. Also, 2 weeks post 2nd vaccination and 8 weeks post challenge, the P% were 100% for both G1 and G3 while 0% in G4.

The results of challenge test of chickens with virulent strain of *P. multocida* type (A) revealed P% 100% for G3, 93% for G1 and 0% for G4 (Table 7). Moreover, challenge test of chickens with virulent strain of *P. multocida* type (D) revealed P% 100% for both G1 and G3 and 0% for G4 (Table 8). Chickens challenged with virulent strain of *M. gallisepticum* showed P% of 80% in G2 and 93% in G3 compared with 0% in G4 (Table 9).

## DISCUSSION

The cellular immune response of chickens that vaccinated with different bacterins was evaluated by H/L ratio. The results indicated that there were significant differences ($p \leq 0.05$) between the vaccinated groups (G1, G2 and G3) and the control one (G4), and also between G1 and G3 but no difference between G2 and G3. These data agreed with Gaunson et al. (2006) who reported that *M. gallisepticum* vaccine activated cellular immune responses in tracheal mucosa including natural killer and cytotoxic T cells responses that are important for the immunity. Also, Abbas et al. (2007) stated that *M. gallisepticum* vaccine induced specific immune responses in vaccinated birds in the form of production of specific antibodies and non-specific factors/cytokines particularly interferon Gamma that activate antigen stimulated B cells, macrophages, cytotoxic T- cells and NK cells. Moreover, Kreslavsky et al. (2012) and Suling et al. (2012) explained that the formaldehyde inactivated Montanide ISA70 based *M. gallisepticum* vaccine causes irritation at inoculation site and induces granuloma formation/development of lymphoid tissues, where the macrophages or antigen presenting cells in the granuloma ingest the microbial antigen from oily suspension and present the microbial protein on their surface in association with self MHC II. Also, the T helper cells of the vaccinated birds recognize their specific antigens on surface of antigen presenting cells and undergo the process of blast formation, proliferation and differentiation into effectors and memory T lymphocytes. Concerning *P. multocida*, Harper et al. (2016) reported that lipopolysaccharide is a primary stimulator of the host immune response and a critical determinant of bacterin protective efficacy.

The humoral immune response of chickens vaccinated with *P. multocida* bacterin as well as combined *P. multocida* and *M. gallisepticum* bacterin was evaluated by IHA. There were significant differences ($p \leq 0.05$) in antibodies titers between the vaccinated groups (G1 and G3) and the control one (G4) regarding *P. multocida* types (A and D). However, significant difference ($p \leq 0.05$) in antibodies titers between G1 and G3 was found in *P. multocida* type (A) not type (D). These findings were parallel with Ahmed et al. (2010) and Abdel-Aziz et al. (2015) who concluded that inactivated FC vaccine adjuvanted with Montanide ISA-70-VG induced early and high immune response with long duration measured by IHA test. It was estimated that prepared *P. multocida* vaccines containing oil adjuvant Mantonide ISA70, ISA774 and W/O emulsion based on tween/span produced high immune response in 4 and 8 weeks old chickens (Belloc et al., 2008).

The humoral immune response of vaccinated chickens with different *M. gallisepticum* bacterins was evaluated by HI. It was found that there were significant differences ($p \leq 0.05$) in antibodies titers between the vaccinated groups (G2 and G3) and the control one (G4), while no differences between G2 and G3. These results are in the same manner with Barbour and Newman (1990) who recorded significant immunoglobulin response specific to *M. gallisepticum* in the sera of chickens collected 3 weeks after the 1st and 2nd vaccination with oil-emulsion vaccine. The potency of the prepared bacterins was evaluated by passive mouse protection test against challenge with virulent strains of *P. multocida* types (A and D) in chickens vaccinated with *P. multocida* bacterin and combined *P. multocida* and *M. gallisepticum* bacterin. The P% post the 2nd vaccination and 8 weeks post challenge was 100% in G3. Similarly, El-Bayomy and Daoud (2004) found an elevation in the protective values of FC adjuvanted vaccines against challenge with virulent strains of *P. multocida* types (A and D). Moreover, Youssef and Tawfik (2011) concluded that inactivated *Pasteurella* vaccine adjuvanted with Montanide ISA50 induced 3.85 and 3.69 log protection in mice against challenge with rabbit *P. multocida* types (A and D). Vaccination challenge test for estimation of protective indices is the main for evaluation and quality control of any prepared vaccine (OIE, 2012). The potency of the prepared bacterins was evaluated by challenge of chickens with virulent strains of *P. multocida* types (A and D) and the results revealed that the P% was (100%) in G3. Jabbri and Moazeni Jula (2005), Ahmed et al. (2010), Ievy et al. (2013), Abdel-Aziz et al. (2015), Ali and Sultana (2015) and Akhtar et al. (2016) demonstrated high protection rate of inactivated FC vaccines in chickens.

The results of potency of the prepared bacterins against challenge of chickens with virulent strain of *M. gallisepticum* showed the highest P% (93%) in G3. The same of this result, Bekele (2015) concluded that formalin inactivated Montanide ISA70 based *M. gallisepticum* induced 100% protection manifested by absence of signs and lesions after challenge. Kleven (2008), Ferguson-Noel et al. (2012) and OIE (2012) found that *M. gallisepticum* bacterin was protective as there were significant differences in air sacs, tracheal and ovarian lesions of the vaccinated birds compared to the non-vaccinated controls. Bekele and Assefa (2018) demonstrated that 16 weeks old chickens vaccinated with inactivated oil-emulsion adjuvant (Montanide ISA 70) *M. gallisepticum* did not show clinical signs or post mortem changes (100% protection).

## CONCLUSION

It could be concluded that the locally prepared combined inactivated *P. multocida* and *M. gallisepticum* vaccine elicited good cellular and humoral immune responses as well as high protection of chickens against both diseases.

## ACKNOWLEDGEMENTS

The authors are grateful to Faculty of Veterinary Medicine, Cairo University, Egypt for the supervision and support, as well as Aerobic Bacterial Vaccines Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Egypt and Mycoplasma Department, Animal Health Research Institute, Dokki, Egypt for covering all the expenses of the experiment needed to conduct the work.

## CONFLICT OF INTEREST

All authors have no conflict of interest.

## AUTHORS CONTRIBUTION

Ibrahim FF performed the experiments and wrote the manuscript. El-Jakee J, Abd El-Ghany WA and El-Rawy EM designed the experiments and reviewed the manuscript. Shaker MM and Ibrahim FF designed the experiments and prepared the vaccine. All authors read and approved the final version.

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