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**Original Research Article**

# THE EFFICACY OF VACCINATION OF LAYER CHICKENS WITH INACTIVATED FOWL CHOLERA BACTERIN PREPARED FROM LOCAL EGYPTIAN STRAINS OF *Pasteurella multocida*

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**Abstract:** This study was carried out to evaluate the efficacy of vaccination of layer chickens with inactivated FC bacterin prepared from local Egyptian strains of *Pasteurella multocida* (*P. multocida).* A total of 200 layer chickens were divided into 5 equal groups, 40 for each. At the age of 6 weeks, chickens in groups (A) and (B) were vaccinated with *P. multocida* serotypes A:1 and A:3, respectively, booster doses were given after 3 weeks (9 weeks old) and challenge was done with virulent serotypes A:1 and A:3 at 2 weeks later (11 weeks old). Chickens in groups (C) and (D) were not vaccinated, only challenged with *P. multocida* serotype A:1 and A:3, respectively. Birds in group (E) were kept as non-vaccinated and non-challenged. Blood samples were collected weekly from all groups for humoral immune response. All the birds were kept under observation for signs, mortalities, lesions and re-isolation of challenging organism and for histopathological examination. Results of the mean Enzyme Linked Immuno-Sorbent Assay (ELISA) revealed that the highest level was at 5 weeks post vaccination as the titers reached to 3970 in group (A) and 3905 in group (B). The clinical signs, mortality rate and lesions were mild in the vaccinated birds while severe lesions were in non-vaccinated and challenged birds. The protection rates were 85 % and 80 % in groups (A) and (B); respectively, while 10 % and 20 % in groups (C) and (D); respectively. The re-isolation rates of *P. multocida* after challenge were 95 % and 90 % in non-vaccinated-challenged birds with *P. multocida* serotypes A:1 and A:3; respectively, while they were 25 % and 15 % in vaccinated-challenged groups with *P. multocida* serotypes A:1 and A:3; respectively. Histopathological examination of *P. multocida* vaccinated-challenged birds revealed mild to no microscopic lesions when compared with non-vaccinated challenged chickens. In conclusion, the prepared FC inactivated bacterin from the local Egyptian predominant *P. multocida* serovars proved efficacy and protection of layer chickens.

**Key words:** *Pasteurella multocida;* chickens; immunization; protection; Egypt

## Introduction

Fowl cholera (FC) is a bacterial disease of domestic and wild birds that causes high economic losses including; deaths, weight losses and condemnations (1-3). Mortalities related to FC infection in layer chickens may range from few percentages up to 100 % (4). The incidence of FC is more common in mature layer chickens than young broilers because of age factors (5, 6). Infection with FC is caused by *Pasteurella multocida* (*P. multocida*) micro-organism (7). *P. multocida* is considered as Gram-negative coccobacilli, non-motile and non-spore former and capsulated organism (8).

Vaccination against FC is considered as one of the most important worldwide strategy to decrease the incidence of infection (9, 10). Globally, living and inactivated (bacterin) vaccines are being used to immunize birds against FC (4, 11). Living attenuated vaccines have advantages regarding good protection with long immunity as well as cross-protection against *P. multocida* of different serotypes or surface lipopolysaccharide (LPS) structures (12). However, living attenuated FC vaccines may lack of maintainable and sustainable attenuation methods and/or instability which may

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lead to risk of returning their virulence. Therefore, inactivated FC bacterins are widely used as there is no chance of the vaccines reverted to virulence and cause the disease (13). In this regard, inactivated FC bacterins have comparative advantages over the living vaccines and are thus favored to protect chickens against infection caused by homologous *P. multocida* strains (14).

Bacterins used for prevention of FC provide homologous but not heterologous protection (15). Immunogenic local strains of *P. multocida* should be selected as the ideal strains to prepare effective bacterin (16). There are 16 different serovars of *P. multocida* and the most common types associated with FC outbreaks are serovars 1, 3 and 4. Evaluation and quality control of the efficacy of locally prepared FC bacterin are based mainly on inactivation of *P. multocida* serovars, followed by vaccination and challenge test by which the protective indices are measured.

According to FAO report, Egyptian poultry production systems are varying from rural very small-scale, extensive poultry production to highly intensive systems with over 70,000 birds per house in industrial commercial systems. The meat production or broiler sector has a pyramid structure; with grandparents at the top of the pyramid, the broilers at the bottom and the broiler breeders in between the two. Egypt is 100 % self-sufficient from table eggs as it produces nearly 13 billion commercial table eggs annually.

Some studies have been conducted in Egypt considering the situation of *P. multocida* infection in layer, breeder and broiler chicken flocks. Researches indicated that the most common circulating *P. multocida* serotypes that circulating in Egyptian flocks are types A:1 and A:3 causing severe economic losses in back yard as well as layer and breeder chicken flocks (17-20).

There is available commercial inactivated oil adjuvant bacterin used for vaccination of Egyptian layer and breeder chicken flocks against FC. This bacterin was prepared from local *P. multocida* serotypes A:1 and A:3 strains and used at age of 8-10 weeks and boostered at 16-17 weeks. However, the bacterin doesn't confer complete protection of flocks against *P. multocida* infection. Therefore, from time to another, trials have been done to prepare and test the efficacy of using locally prepared FC bacterin from the predominant circulating *P. multocida* serovars in chickens (21-23).

Accordingly, this work was designed to evaluate the efficacy of vaccination of layer chickens with inactivated FC bacterin prepared from local Egyptian strains of *P. multocida*.

## Materials and methods

### *Experimental design:*

A total of 200, day-old layer chickens was obtained from local hatcheries and reared on thoroughly cleaned and disinfected semi closed houses for 13 weeks. Birds were vaccinated using standard protocol for vaccination. Feed and water was given *ad libitum*. At 6 weeks old chickens, birds were divided into 5 equal groups 40 for each. Groups (A) and (B) were vaccinated with 0.5 ml I/M with *P. multocida* serotypes A:1 and A:3; respectively, booster doses were given after 3 weeks (9 weeks old), and challenge was done orally with virulent serotypes A:1 and A:3 at 2 weeks later (11 weeks old). Chickens in groups (C) and (D) were not vaccinated and challenged with *P. multocida* serotype A:1 and A:3; respectively. Birds in group (E) were kept as non-vaccinated and non-challenged. The experiment was done according to the National Guidelines and Regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC) of Cairo University with approval number (CU II F 100 18).

### *Preparation of inactivated bacterin from the predominant P. multocida local strains*

Local *P. multocida* strains serotypes (A:1 and A:3) that were isolated from different Egyptian governorates were used. The used *P. multocida* strains were previously serologically and molecularly identified (20). Inactivated bacterin was prepared according to method described by Borkowska-Opacka et al. (24). Simply, *P. multocida* strains were grown in brain heart infusion broth at 37°C for 16-24 hr to obtain a dense culture containing approximately $10^8$ CFU of each strain. Formalin was added to the culture in final concentration of 0.2 % and the formalized culture was re-incubated at 37°C for 24 hr to ensure complete bacterial inactivation. Two percent of aluminium hydroxide gel was added in concentration of 20 % and was mixed well with the culture. Cultures were equally mixed

together. The bacterin was standardized to contain $10^8$ CFU/0.5ml dose. Finally, the bacterin was preserved with 0.01 % of thiomersal and stored at 4 C° until use. The quality control parameters of the locally prepared bacterin were sterility, safety and potency test (25). Briefly, the prepared bacterin was inoculated on bacteriological and mycological media to prove its sterility or purity from any bacterial or fungal contaminations. In addition, 0.5 ml of the prepared bacterin was inoculated in 5 chickens and the birds were kept under observation for a weeks to ensure that the bacterin induced no adverse effects like signs, lesions or mortalities. The potency test was applied by collection of blood samples and measuring of immunological parameters.

*Vaccination scheme*

Primary vaccination was done at the age of 6 weeks, 0.5 ml of the prepared inactivated *P. multocida* bacterin containing $10^8$ CFU/0.5 ml was inoculated intramuscular (I/M) into the thigh region in chickens of groups (A) and (B). Booster vaccination was done at 9 weeks of age (3 weeks after primary vaccination) (24).

*Challenge test with virulent P. multocida*

Serovars of *P. multocida* that were used for bacterin preparation were used for chickens challenge. Both vaccinated and non-vaccinated chickens (groups A, B, C and D) were challenged I/M with virulent *P. multocida* serotypes A:1 and A:3 separately at 2 weeks after booster vaccination (11 weeks old) in a dose of 0.1 ml of bacterial suspension/bird containing $10^7$/ml that inoculated I/M (26).

*Parameters for evaluation of inactivated P. multocida bacterin in chickens*

*Immunological parameters:*

Detection of humeral immune response of vaccinated and challenged birds was done using Enzyme Linked Immuno-Sorbent Assay (ELISA). Commercial ELISA kit (ID-VET) was used and the test was done as manufacturer's instructions. Blood samples were collected from the wing vein of birds in each group pre-vaccination and weekly after vaccination till the end of the study to determine the serum antibody titer of chickens. The results of antibody titers were determined from S/P ratio

$$\frac{S}{P} = \frac{\text{(Sample mean – negative control)}}{\text{(positive control-negative control)}}$$

$$\text{Log}_{10}\ \text{Titer} = 1.09\ (\log_{10}\ S/P) + 3.36$$

*Clinical parameters*

The clinical signs, mortality rate and post-mortem lesions specific for *P. multocida* were recorded in groups two weeks post challenge for measuring bacterin protection rate.

$$\text{The Protection rate (\%)} = \frac{\text{(Number of living birds)}}{\text{(Total number of birds )}}$$

*Re-isolation rate*

Liver was collected from dead as well as sacrificed living birds at the end of the study in each group for *P. multocida* re-isolation. It was done by inoculation of the liver on blood agar media and then by morphological and biochemical identification (27-29).

*Histopathological examination*

Three chickens from each group were sacrificed by cervical dislocation at the end of study (13 weeks old) for histopathological examination and tissue specimens were collected from heart, liver and spleen and then fixed in 10% formol saline solution. After proper fixation, the specimens were dehydrated in ascending grades of ethanol from 70 % to 100 %, diluted in alcohol (methyl, ethyl and absolute ethyle), cleared in xylol and manually embedded in paraffin at 56°C in hot air oven for 24 hr. Thin tissue sections, 5 µm thick were cut from paraffin blocks and stained with Hematoxylin and Eosin (HE) for histopathological examination through the light microscope (30).

## Results and discussion

Fowl cholera is one of the most important problem facing poultry industry, so vaccination against the disease is practiced as preventive measures in many countries of the world including Egypt (31). Both live and inactivated *P. multocida* vaccines had been attempted to prevent the disease (4). Inactivated *P. multocida* vaccines are widely

used as the organisms do not have chance to be reverted to virulence to cause the disease (13).

In the present work, an inactivated local bacterin was prepared using *P. multocida* field strains and its efficacy was determined. The quality control assessment of the prepared *P. multocida* bacterin showed it was sterile and free from any bacterial or fungal contaminations and it was safe as it didn't produce any local or systemic reactions as well as no mortalities in inoculated birds.

Table (1) shows the potency of the locally prepared *P. multocida* bacterin through determining the humoral immune response using ELISA. In both groups (A) (vaccinated and challenged birds with *P. multocida* type A1) and (B) (vaccinated and challenged birds with *P. multocida* type A3), the mean ELISA antibody titers increased from (80) pre-vaccination level to reach (2260) and (2010) at the 3rd week after primary vaccination; respectively, however, two weeks after secondary vaccination, the antibody titers reach (4350) and (3980) respectively, then declined to (2998) and (2679) one week after challenge then increased to (3970) and (3905) at two weeks after challenge, respectively. Results of the mean ELISA antibody levels in controls groups (C) (non-vaccinated and challenged birds with *P. multocida* type A:1) and (D) (non- vaccinated and challenged birds with *P. multocida* type A:3) were 60-80 before challenge and then increased to (95) at two weeks after challenge. Group (E) (non-vaccinated-non challenged control) showed steady mean ELISA antibody levels (65-80). Solano et al. (32) developed ELISA assay to determine the humoral immune response of chicken to *P. multocida* and compared the results with indirect haemagglutination (IHA) test as they concluded that the antibody titers measured by ELISA was at least twice as sensitive as IHA. Furthermore, Avakian et al. (33), Perelman et al. (9) and Esmaily et al. (34) recorded that polyvalent FC oil-based bacterin induced a high antibody titer in broiler breeder hens measured by ELISA technique. In addition, Jabbri and Moazeni Jula (35) stated that inactivated trivalent FC vaccine consisted of serotypes 1, 3 and 4 *P. multocida* strains induced immunogenic response in vaccinated chickens, as ELISA assay showed a considerable increase in antibody titer after twice vaccination of 8 weeks old chickens. Birds vaccinated two or three times between the ages of 7 and 20 weeks became sufficiently immune tolerated to FC challenge, while those were not vaccinated or only vaccinated at the age of 7 weeks were not sufficiently immunized using ELISA (36). The results of humoral immune response obtained in this study were comparable Akhtar et al. (16) who tested a formalin killed FC in 15 weeks old chickens and found an increase in humoral antibody titers after booster vaccination.

The bird's immune response to *P. multocida* bacterin was previously explained (37, 38). The capsule and LPS of *P. multocida* cell surface are considered primary stimulators of immune response and critical determinants of bacterin

**Table 1:** Mean ELISA antibody titres of sera in chicken groups pre-vaccination; one, two and three weeks after primary vaccination; one and two weeks after secondary vaccination and one and two weeks after challenge

| Group | Treatment | *P. multocida* strain | *P. multocida* mean antibody titers/ weeks after vaccination | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Weeks after primary vaccination (6th wks old) | | | Weeks after secondary vaccination (9th wks old) | | Weeks after challenge (11th wks old) | |
| | | | Pre-V* | 1 | 2 | 3 | 1 | 2 | 1 | 2 |
| A | Vaccinated- challenged | A:1 | 80 | 687 | 1760 | 2260 | 3400 | 4350 | 2998 | 3970 |
| B | | A:3 | 80 | 654 | 1590 | 2010 | 3085 | 3980 | 2676 | 3905 |
| C | Non vaccinated-challenged control | A:1 | 80 | 70 | 70 | 65 | 70 | 80 | 95 | 95 |
| D | | A:3 | 80 | 75 | 60 | 60 | 75 | 70 | 90 | 95 |
| E | Non vaccinated- non challenged control | – | 70 | 70 | 80 | 75 | 65 | 80 | 75 | 65 |

Pre V*: Pre-vaccination

protective efficacy (39). Both play key roles in a range of interaction between the bacteria and the hosts they colonize or infect. Gong et al. (40) demonstrated that outer membrane proteins (H and A) of *P. multocida* are the major immunogene antigens which play an important role in confer resistance against infections. The Omps promote adherence to host cell surfaces and are therefore likely involve in *P. multocida* virulence (41).

Our results revealed that, the clinical signs of *P. multocida* vaccinated and challenged chicken groups were mild depression, off food, diarrhea, septicaemia and congested mucous membrane of conjunctiva and buccal cavity. Severe signs were observed in non-vaccinated challenged controls groups, while no signs appeared in the non-challenged control group during the observation period. These results were in agreement with Levy et al. (42) who recorded signs of depression dullness, anorexia, greenish diarrhea and labored breathing in *P. multocida* challenged non vaccinated chickens, while the vaccinated birds did not show clinical signs except dullness and depression. Also, signs of FC in commercial layer flocks were recorded as depression, anorexia with ruffled feathers, mucous discharge from mouth and nares and cyanotic comb and wattles (43).

The mortality rates in different groups that recorded here were 15 % and 20 % in vaccinated and challenged birds with *P. multocida* type A:1 and A:3; respectively, however, in non-vaccinated and challenged controls, they were 90 % and 80 % for *P. multocida* type A:1 and A:3; respectively (Table 2). These results were in a partial agreement with others (44, 45). The results showed that vaccinated chickens were resistant to challenge with *P. multocida* A:1 and A:3 strains, where the protection rates were 85 % and 80 % respectively, while they were 10 % and 20 % in the non-vaccinated-controls, respectively (Table 2). Two doses of prepared bacterin induced good protection (80-90 %) against challenge with *P. multocida* of homologous immunogenic type but low protection (10-30 %) against heterologous challenge (46). An Egyptian study concluded that locally prepared polyvalent bacterins should be used in cases of FC outbreaks, and the capsular antigen plays a little role in immunization when compared with the somatic antigen (47). Inactivated trivalent FC vaccine consists of serotype 1, 3 and 4 *P. multocida* provided 70-80 % protection in chickens against challenge with homologous strains (35). Also, some other Egyptian trials revealed that adjuvented local FC vaccine gave 100 % protection in chickens against challenge with virulent strains of *P. multocida* types A and D (48) as well as 95 % and 90 % for types A:5 and D:2; respectively (49). Furthermore, the protection rate was 100 % in chickens vaccinated twice with alum-precipitated FC vaccine (50).

Post-mortem examination of chickens revealed mild and severe lesions in *P. multocida* vaccinated -challenged and non-vaccinated-challenged control groups, respectively. The lesions included septicaemia, congestion of internal organs, enlarged liver with sub-capsular hemorrhage, pericarditis and enlarged and congested spleen. These lesions were in accordance with other study (45) where the lesions of birds vaccinated with double doses of *P. multocida* local bacterin were congested heart and

**Table 2:** Results of protection rates in different chicken groups after vaccination with local *P. multocida* bacterin and challenge with the same serovars

| Group | Chicken groups | Challenge *P. multocida* Strain | No. of chickens | No. of survived birds | No. of dead birds | Mortality rate (%) | Protection Rate (%) |
|---|---|---|---|---|---|---|---|
| A | Vaccinated-challenged | A:1 | 40 | 34 | 6 | 15 | 85 |
| B | | A:3 | 40 | 32 | 8 | 20 | 80 |
| C | Non vaccinated-challenged control | A:1 | 40 | 4 | 36 | 90 | 10 |
| D | | A:3 | 40 | 8 | 32 | 80 | 20 |
| E | Non vaccinated-non challenged control | _ | 40 | 40 | 0 | 0 | 100 |

slight congestion of the liver and spleen, however the lesions in non-vaccinated-challenged birds were congestion of the subcutaneous blood vessels, dark red muscles, enlarged and congested liver and spleen and pericarditis. In other study, severe congestion, hemorrhages, pericardial and peritoneum exudations, enlargement of spleen and liver and white necrotic foci over liver were reported in chickens vaccinated with type A:1 of *P. multocida* at 14 day post-challenge (46).

Table 3 reveals that *P. multocida* could be re-isolated from liver, heart blood and spleen of the chickens with high rate (90-95 %) and low rate (15-25 %) in non-vaccinated and vaccinated-challenged birds; respectively. These findings supported by Egyptian researcher Mahmoud (51) who found that the incidence of isolation of *P. multocida* was higher from non-vaccinated flocks than those from vaccinated ones. Partial agreement with ours revealed that *P. multocida* could not be recovered from immunized and challenged survived chickens, while it could be isolated from all dead or diseased birds (35).

In this study, Table 4 and Figures 1-4 demonstrate the histopathological findings in different examined organs at the end of the experiment. Figure 1 shows the microscopic lesions of chickens vaccinated and challenged with *P. multocida* serotype A:1. Mild congestion of hepatic central vein and some inflammatory cells infiltrate (A), normal myocardium (B) and congested red pulp of spleen (C) were the observed lesions. In chickens vaccinated and challenged with *P. multocida* serotype A:3 (fig. 2) showed hydropic degeneration of hepatocytes (A), slight congestion of the myocardium (B) and hyperplasia of splenic lymphoid follicle (C). The previous results were in a partial agreement who noticed congestion with presence of some degenerative changes of liver, mild depletion of splenic lymphoid cells and slight myocarditis in *P. multocida* vaccinated and challenged chickens (45, 52).

**Table 3:** Results of re-isolation of *P. multocida* among chicken groups after vaccination with local *P. multocida* bacterin and challenge with the same serovars

| Group | Chicken groups | Challenge *P. multocida* Strain | No. of chickens | Re-isolation % of *P. multocida* |
|---|---|---|---|---|
| A | Vaccinated- challenged | A:1 | 40 | 6/40 (15%) |
| B | | A:3 | 40 | 10/40 (25%) |
| C | Non vaccinated-challenged control | A:1 | 40 | 38/40 (95%) |
| D | | A:3 | 40 | 36/40 (90%) |
| E | Non vaccinated-non challenged control | _ | 40 | 0/40 (0%) |

**Table 4:** Results of histopathological examination of chicken groups after vaccination with local *P. multocida* bacterin and challenge with the same serovars

| Point of comparison | Group | | | |
|---|---|---|---|---|
| | A | B | C | D |
| **Liver** | | | | |
| Congestion of central vein | + | - | - | + |
| Inflammatory cells infiltrate | + | - | + | - |
| Coagulative necrosis | - | - | + | - |
| Hydropic degeneration | - | + | - | + |
| **Myocardium** | | | | |
| Congestion | - | + | - | - |
| Inflammatory cells infiltrate | - | - | + | - |
| Endocardial haemorrhages | - | - | - | + |
| **Spleen** | | | | |
| Red pulb congestion | + | - | + | - |
| Lymphoid follicle hyperplasia | - | + | - | + |
| Focal area of necrosis | - | - | - | + |

+ = Present - = Absent

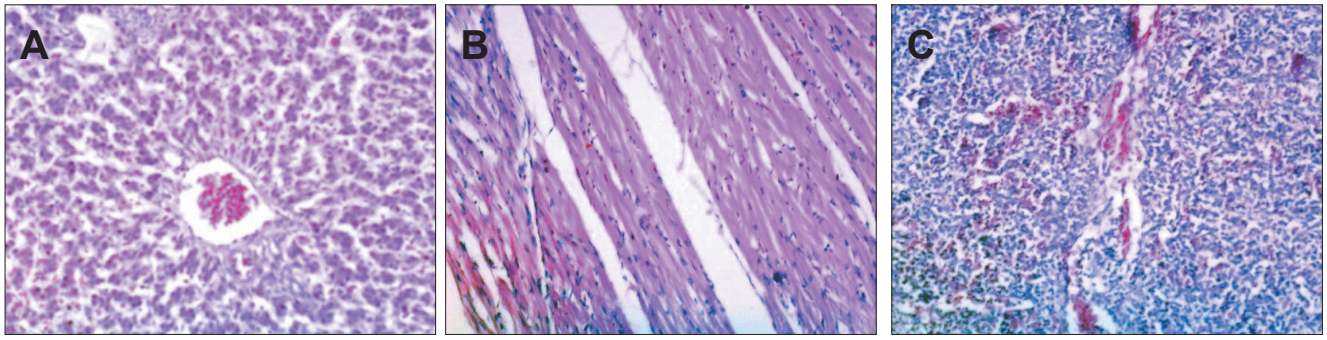


**Figure 1:** Chickens vaccinated and challenged chickens with *P. multocida* serotype A:1 (group A) (H&E. X 200): A: Liver shows mild congestion of central vein and some inflammatory cells infiltrate, B: Heart showing normal myocardium, C: Spleen showing congested red pulp

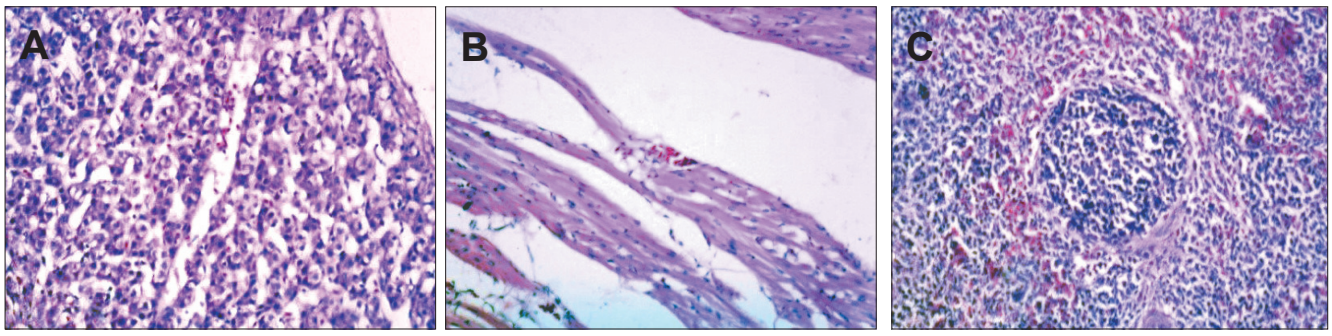


**Figure 2:** Chickens vaccinated and challenged chickens with *P. multocida* serotype A:3 (group B) (H&E. X 200): A: Liver showing hydropic degeneration of hepatocytes, B: Heart shows slight congestion of the myocardium, C: Spleen showing hyperplasia of lymphoid follicle

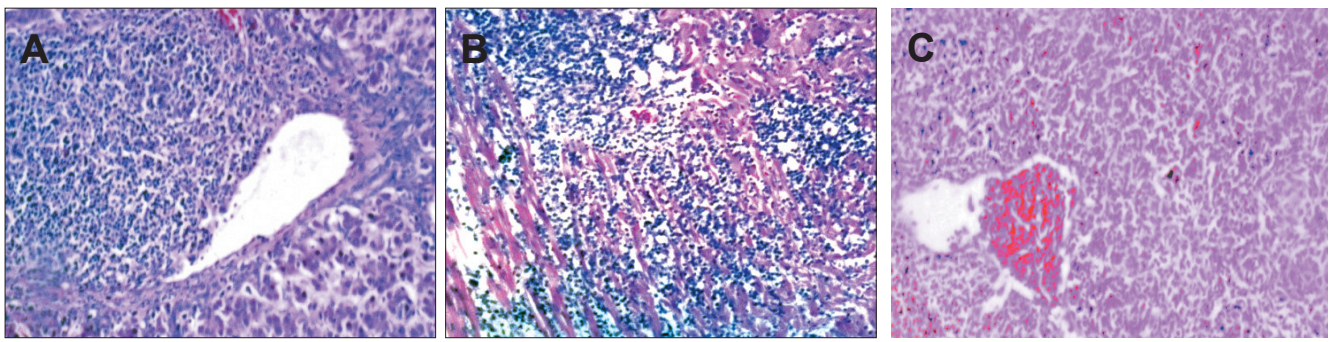


**Figure 3:** Chickens experimentally challenged with *P. multocida* A:1 strain (group C) (H&E. X 200): A: Liver showing area of coagulative necrosis infiltrated with heterophils, B: Heart showing severe inflammatory cells infiltration of myocardium, C: Spleen shows congestion of red pulp and splenic artery

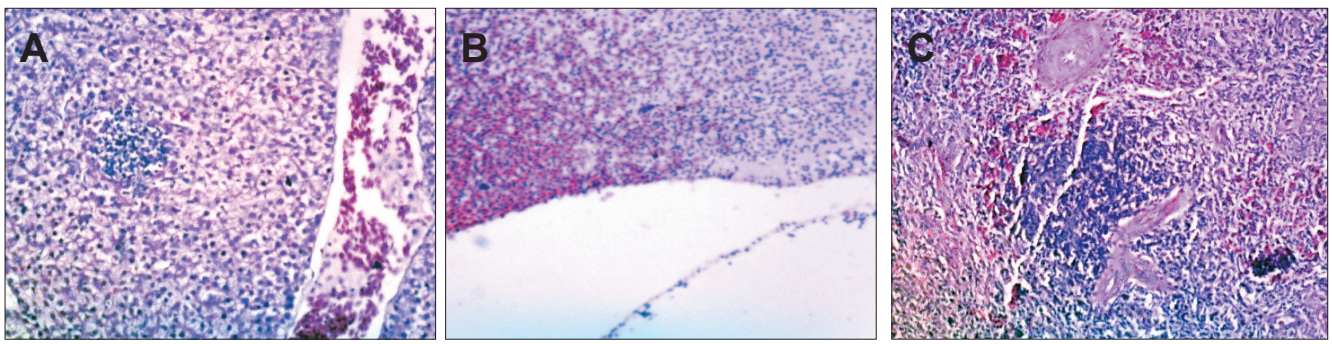


**Figure 4:** Chickens experimentally challenged with *P. multocida* A:3 strain (group D) (H&E. X 200): A: Liver showing congestion of portal vein and hydropic degeneration of cytoplasm, B: Heart sub endocardial haemorrhage with heterophilic infiltration, C: Spleen shows severe congestion and focal area of necrosis (arrow)

Histopathological lesions in chickens challenged with *P. multocida* A:1 strain (fig. 3) showed area of hepatic coagulative necrosis infiltrated with heterophils (A), severe inflammatory cells infiltration of myocardium (B) and congestion of splenic red pulp and artery (C). Moreover, in chickens experimentally challenged with *P. multocida* A:3 strain (fig. 4), the liver showed congestion of portal vein and hydropic degeneration of cytoplasm (A), the heart revealed sub endocardial haemorrhage with heterophilic infiltration (B) and the spleen demonstrated severe congestion and focal area of necrosis (C). Similarly, histopathological changes of local isolates of *P. multocida* A:1 in chickens were congestion, hemorrhages and mild degeneration of liver associated with necrotic changes involving groups of hepatic parenchymatous cells with prominent heterophilic infiltration (53, 54).

These lesions could be attributed to the direct effect of the endotoxin and ischemia which resulted from the bacterial emboli.

From the above mentioned results, it could be concluded that the prepared FC inactivated bacterin from the local Egyptian predominant *P. multocida* serovars proved efficacy and protection of layer chickens.

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The authors declare that they have no conflict of interest.

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## UČINKOVITOST CEPLJENJA KOKOŠI NESNIC Z INAKTIVIRANO BAKTERIJO KOLERE PERJADI, PRIPRAVLJENE IZ LOKALNIH EGIPTOVSKIH SEVOV BAKTERIJE *Pasteurella multocida*

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**Povzetek:** Raziskava je bila izvedena z namenom ocenitve učinkovitosti cepljenja kokoši nesnic z inaktivirano bakterijo FC, pripravljeno iz lokalnih egiptovskih sevov bakterije *Pasteurella multocida (P. multocida).* Skupno 200 kokoši nesnic je bilo razdeljenih v 5 enakih skupin. V vsaki skupini je bilo 40 kokoši. Pri 6 tednih smo kokoši v skupinah A in B cepili s serotipoma *P. multocida* A:1 in A:3, po 3 tednih, ko so bile živali stare 9 tednov, so dobile poživitvene doze cepiva. Po dveh tednih (v starosti 11 tednov) so bile kokoši okužene z virulentnima serotipoma A:1 in A:3. Piščanci v skupinah C in D niso bili cepljeni temveč samo okuženi s serotipoma A:1 in A:3. Kokoši v skupini E niso bile niti cepljene, niti okužene. Vzorci krvi so bili odvzeti pri vseh skupinah tedensko za preverjanje humoralnega imunskega odziva. Vse kokoši smo stalno opazovali in beležili prisotnost bolezenskih znakov, različnih ran in umiranje kokoši. Pri poginulih kokoših smo osamili bakterije ter opravili histopatološki pregled. Rezultati encimsko-imunskega testa (ELISA) so pokazali da je bila najvišja stopnja zaščite dosežena 5 tednov po cepljenju, saj so titri dosegli 3970 v skupini A in 3905 v skupini B. Klinični znaki, stopnja umrljivosti in rane so bili pri cepljenih kokoših blagi, hude rane pa so bile vidne pri necepljenih in okuženih kokoših. Stopnja zaščite je bila v skupinah A in B 85- oziroma 80-odstotna, v skupinah C in D pa 10- oziroma 20-odstotna. Stopnje ponovne izolacije *P. multocida* po okužbi so bile 90 in 95 odstotkov pri kokoših, ki niso bile cepljene, medtem, ko so bile v skupinah, ki so bile okužene s *P. multocida* serotipa A:1 in A:3 15- in 25-odstotkov. Histopatološki pregled cepljenih in okuženih kokoši je pokazal popolno odsotnost ali prisotnost blagih mikroskopskih poškodb, medtem ko so imele necepljene okužene kokoši bolj obsežne histopatološke poškodbe. Pripravljena inaktivirana bakterija FC iz lokalnih egiptovskih prevladujočih serovarov *P. multocide* se je izkazala za učinkovito zaščito kokoši nesnic.

**Ključne besede:** *Pasteurella multocida*; kokoši; imunizacija; zaščita; Egipt