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Preparation, experimental and molecular evaluation of fowl cholera chicken embryo derived inactivated bacterin

Shimaa M.A. Khair[1], Tolba Y. Abdelmottileb[2], Mostafa A. Shahata[2], Ahmed K. Hassan[2*]

[1]*Department of Poultry and Rabbit Medicine, Faculty of Veterinary Medicine, Sphinx University, Assiut, Egypt.*
[2]*Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.*

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***Correspondence:**

Corresponding author: Ahmed K. Hassan
E-mail address: ahassan@aun.edu.eg

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ABSTRACT

Fowl cholera is a contagious bacterial disease of poultry caused by *Pasteurella multocida* and has a significant economic importance worldwide. Fowl cholera prevention depends mainly on vaccination using live and inactivated vaccines, but they have many limitations. The present study aimed to prepare fowl cholera chicken embryo derived inactivated bacterin and to evaluate it by experimental infection and molecular evaluation. Fowl cholera chicken embryo derived inactivated bacterin prepared by *in-vivo* growing of *Pasteurella multocida* serotype 3 (P-1059) on chicken embryonating eggs, then inactivated by formalin (0.3%) and adjuvant added and then quality control parameters tested. The prepared bacterin was evaluated by experimental challenge with homologues and heterologous serotypes of *Pasteurella multocida* serotypes in comparison with commercial inactivated multivalent vaccine. Molecular evaluation of prepared bacterin was carried out using SDS-PAGE for *in-vivo* and *in-vitro* grown *Pasteurella multocida* serotypes. Results revealed that the prepared fowl cholera chicken embryo derived inactivated bacterin was free from bacterial and fungal contaminations and safe for use. Fowl cholera chicken embryo derived inactivated bacterin provided high protection rates with low mortalities against experimental infections with homologues and heterologous serotypes of *Pasteurella multocida*. Results of SDS-PAGE revealed that *in-vivo* grown *Pasteurella multocida* serotypes showed expression of additional specific bands (35kDa, 39kDa). It was suggested that 39kDa is a dominant structural protein in *Pasteurella multocida* grown *in-vivo* and play a role in antigenic cross protection among its serotypes. In conclusion, Fowl cholera chicken embryo derived inactivated bacterin is effective against infections of homologous and heterologous *Pasteurella multocida* serotypes.

Introduction

Fowl cholera is a contagious bacterial disease of poultry and wild birds (Bisgaard *et al*., 2005; Marza *et al*., 2015; Xiao *et al*., 2015; Blackall and Hofacre, 2020) causing severe economic losses in broiler breeders (Zhang *et al*., 2004), free-range layers (Singh *et al*., 2013), organic broilers (Singh *et al*., 2014), backyard birds (Christensen *et al*., 1998), turkey and the duck industries (Christensen and Bisgaard, 2000), and wild birds (Friend, 1999). Economic losses due to fowl cholera include mortalities, weight losses and condemnations, in addition to drop of egg production in laying hens (Glisson *et al*., 2008; OIE, 2008; Chrzastek *et al*., 2013). Fowl cholera infection is caused by *Pasteurella multocida* (*P. multocida*) which is Gram-negative coccobacilli, capsulated, non-spore forming and non-motile micro-organism (Eigaard *et al*., 2006; Purushothaman *et al*., 2008). Fowl cholera characterized by septicemic picture, with high rates of morbidity and mortality in avian species especially chicken, turkey, and ducks. Its clinical signs include acute cases which are usually appeared for few hours before death; signs in chicken are fever, ruffled feathers, mucoid nasal and oral discharges, and comb and wattles are cyanosed (Glisson *et al*., 2008). Chronic localized conditions of fowl cholera may occur affecting wattles, sinuses, joints, foot pads, and sternal bursae, and torticollis sometimes occurs (Blackall and Hofacre, 2020).

Vaccination against fowl cholera is the most common preventive measure to reduce the incidence of the disease worldwide (Perelman *et al*., 1990; Kardos *et al*., 2007). Living vaccines and inactivated bacterins have been used to immunize birds against fowl cholera as a major procedure to control the disease (Rhoades and Rimler, 1991), but both have many limitations (Glisson *et al*., 2013). Inactivated bacterins usually contain whole cells of Heddleston serovars 1, 3, 4 and 3/4 emulsified in an oil adjuvant. They are effective only against challenge with homologues serotypes of *P. multocida* (Heddleston *et al*., 1970; Ibrahim *et al*., 2002; Harper *et al*., 2015), but they are safe in use and do not lead to outbreaks of fowl cholera (OIE, 2012; Abd El-Ghany *et al*., 2020). Three live vaccines of fowl cholera are available for use, are CU, a strain of low virulence; M-9, a mutant of CU with very low virulence; and PM-1, a mutant of CU intermediate in virulence between CU and M-9. Live vaccines are able to provide long immunity and suitable cross-protection against different serotypes of *P. multocida* (Heddleston *et al*., 1972; Hofacre *et al*., 1986; Ibrahim *et al*., 2002; Harper and Boyce, 2017) but may lead to outbreaks of fowl cholera (Bierer and Derieux, 1972; Ibrahim *et al*., 2002; Harper *et al*., 2016).

Pasteurella multocida usually is grown *in-vitro* using artificial media as brain heart infusion broth and agar. The growth of *P. multocida* strains *in-vivo* (chicken embryo) instead of *in-vitro* resulted in expression of additional antigens, which may lead to demonstration of antigenic cross-reactivity among homologous and heterologous serotypes (Ibrahim *et al*., 2002).

The present research aimed to prepare fowl cholera chicken embryo derived inactivated bacterin, and to evaluate the prepared vaccine by experimental infection and molecular evaluation.

Materials and methods

Preparation of bacterial strains

Bacterial strains including *P. multocida* serotype 3 (P-1059) and *P.*

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multocida serotype 4 (P-1662) obtained from Prof. Dr. Ragab Sayed Ibrahim (Ibrahim *et al*., 2002) were diluted with buffer saline and then inoculated into brain heart infusion (BHI) broth (Interchim, Life Science, 211 bis, avenue JF Kennedy, BP 1140 -031000 Montlucon, France) overnight at 37ºC. Subculture was done into BHI agar (Interchim, Life Science, 211 bis, avenue JF Kennedy, BP 1140 -031000 Montlucon, France) plates for 24-48 hours at 37ºC and then examined for growth of *P. multocida* individual colonies.

Inoculation of bacterial strains into embryonating chicken eggs

Twenty-five embryonating chicken eggs (Faculty of Agriculture, Assiut University) were divided into five equal groups, each group includes five embryonating chicken eggs. In first group, chicken embryonating eggs were inoculated with 0.1 ml BHI broth containing *P. multocida* serotype 3 (P-1059) via allantoic sac at 7-9 days of age. In second group, chicken embryonating eggs were inoculated with 0.1 ml BHI broth containing *P. multocida* serotype 3 (P-1059) via yolk sac at 5-7 days of age. In third group, chicken embryonating eggs were inoculated with 0.1 ml BHI broth containing *P. multocida* serotype 4 (P-1662) via allantoic sac at 7-9 days of age. In fourth group, chicken embryonating eggs were inoculated with 0.1 ml BHI broth containing *P. multocida* serotype 4 (P-1662) via yolk sac at 5-7 days of age. In the fifth group, chicken embryonating eggs were inoculated with 0.1 ml sterile BHI broth via allantoic sac and kept as control. Inoculated eggs were incubated at 37ºC and were turned at 45 degree 3 times and examined daily by candling for embryonic mortality. After embryonic death, embryonic fluids were harvested and stored at deep freeze (-80ºC).

Preparation of fowl cholera chicken embryo derived inactivated bacterin

Fowl cholera chicken embryo derived inactivated bacterin was prepared from *P. multocida* serotype 3 (P-1059). Concentration of *P. multocida* serotype 3 (P-1059) in embryonic fluids were adjusted to be 10^7 CFU/ ml by colony forming unit technique. Preparation of bacterin was done according to OIE Terrestrial Manual (2021), briefly; harvested allantoic fluids inactivated by addition of formalin 0.3% and then mixed well and incubated overnight at room temperature to allow complete bacterial inactivation. Equal volumes of bacterial suspension and Freund's adjuvant (water-in-oil emulsion, containing killed Mycobacterium cells, Thermo Fisher Scientific, Waltham, Massachusetts, USA) were mixed. The bacterin was standardized to contain 10^7 CFU/ 0.5 ml dose. The quality control parameters of the prepared bacterin including sterility and safety tests were done according to Code of American Federal Regulation (1985). The sterility of the bacterin was determined at each stage of production, this was achieved by culturing on bacteriological and mycological media and microscopic examination to prove its sterility from bacterial or fungal contaminations. For safety test, 0.5 ml of the prepared bacterin was inoculated in 5 chickens and the birds were kept under observation for a week to ensure that the bacterin induced no adverse effects like clinical signs, lesions, or mortalities.

Evaluation of prepared bacterin by experimental infection

One hundred and five, one-day old chicks (Ross, Assiut Company for Development, and Investment, Alkossia, Assiut, Egypt) were randomly divided into seven equal groups, each group includes fifteen chicks as the following: group one: vaccinated with prepared bacterin and infected with homologues *P. multocida* serotype 3 (P-1059), group two: vaccinated with prepared bacterin and infected with heterologous *P. multocida* serotype 4 (P-1662), group three: vaccinated with commercial vaccine (SERVAC inactivated Fowl cholera Vaccine, containing *P. multocida* serotypes 1,3, 3/4, 4; Veterinary Serum and Vaccine Research Institute, El-Sekka El-Beda st., Abbasia, Cairo, Egypt) and infected with *P. multocida* serotype 3 (P-1059), group four: vaccinated with commercial vaccine and infected with *P. multocida* serotype 4 (P-1662), group five: not vaccinated and infected with *P. multocida* serotype 3 (P-1059), group six: not vaccinated and infected with *P. multocida* serotype 4 (P-1662), group seven: not vaccinated and not infected (control). Throughout the experimental period, the chickens were kept in cleaned and disinfected isolated rooms (Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt) and care with provided as required by Institutional Animal Care Committee. First dose of vaccination was administrated by subcutaneous injection (0.5 ml) into chickens at the 14th day of age as follow; chickens of first and second groups were vaccinated with prepared bacterin, chickens of third and fourth groups were vaccinated with commercial vaccine. Second dose of vaccination was applied at 28th day of age with the same vaccines, same dose, and same method of administration. Challenge with bacterial strains was done at the 35th day of age by subcutaneous injection as follow: 0.1 ml BHI broth containing 10^9 CFU/ml of *P. multocida* serotype 3 (P-1059) was injected into chickens of groups one, three, and five; and 0.1 ml BHI broth containing 10^9 CFU/ml of *P. multocida* serotype 4 (P-1662) was injected into chickens of groups two, four, and six. Chickens were observed for mortality, appearance of clinical symptoms and postmortem lesions specific for *P. multocida* infection until the end of experiment (42nd day of age). Protection rates were measured in different groups.

Molecular evaluation of prepared bacterin by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

In-vitro and *in-vivo* cultures of bacterial strains; including *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1662), which propagated on both BHI (agar and broth) and chicken embryonating eggs (via allantoic and yolk sacs) were harvested and then diluted with equal amounts of 4X sample buffer solution and placed on a boiling water-bath for 10 minutes. The mixtures were then centrifuged at 16000 rpm for 5 minutes, and supernatants (protein) were collected for electrophoresis. Vertical gel electrophoresis was carried out by the application of 20 µl of protein per lane in vertical electrophoresis cell according to the method of Laemmli (1970) using 1X running buffer solution. It was performed at 100 V for 2 hours in 5% stacking gel and 12% resolving gel. Approximate molecular weights were determined by application of 5 µl pre-stained protein marker in lane No. one. After gel electrophoresis, gel was subjected to staining with Coomassie blue R-250 (Sigma-Aldrich) overnight on shaker, and then de-stained using de-staining solution overnight on shaker.

Results

Preparation of fowl cholera chicken embryo derived inactivated bacterin

The prepared formalin (0.3%) inactivated allantoic fluid (bacterin) containing *P. multocida* serotype 3 (P-1059) was checked for quality control parameters, and results showed that it is free from any bacterial or fungal contaminations. Results of the safety test for the prepared bacterin pointed out that it is safe and did not produce any local or systemic reactions as well as no mortalities in the five inoculated birds.

Evaluation of prepared bacterin by experimental infection

During observation of chickens after bacterial infections (35th day of age), clinical signs appeared 3 days post infection, sudden death occurred in non-vaccinated challenged chickens of groups five and six. Other clinical sings include depression, decreased feed and water intakes, whitish diarrhea and respiratory symptoms as cough, rales, nasal and ocular discharges, and facial edema.

Postmortem examination of dead chickens showed that, some birds

dead fleshy and in good condition, there was generalized congestion all over the body. Internal examination reveals septicemic picture, liver and spleen were enlarged, congested and hemorrhagic, kidneys were swollen and congested, also there was congestion and exudates in trachea and bronchi, lungs were edematous and congested, intestine showing enteritis and pancreas was enlarged, congested and hemorrhagic (Figure 1). Clinical signs and postmortem lesions varied according to challenge and vaccination. Clinical signs and postmortem lesions were severe in non-vaccinated infected birds and mild to moderate in vaccinated infected birds. Chickens of control group showed no clinical signs, and no postmortem lesions. Results of clinical signs and postmortem lesions are summarized in Table 1.

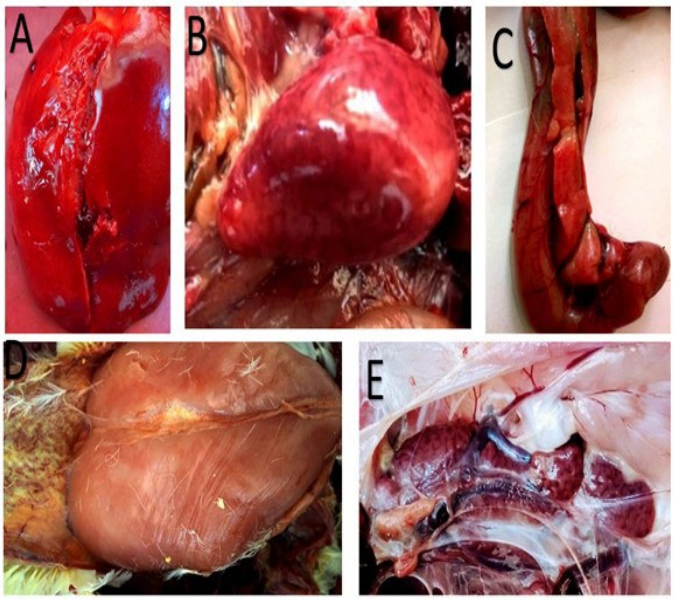

Fig. 1. Postmortem lesions in chickens non-vaccinated, experimentally infected with *P. multocida*; A: liver, B: spleen, C: duodenum and pancreas, D: generalized congestion and, E: kidneys.

The mortality rates in different groups were recorded and results were as follow; mortality rate was 13.3% in group one, in which chickens were vaccinated with prepared vaccine and infected with homologues *P. multocida* serotype 3 (P-1059), and it was 20% in group two, in which chickens were vaccinated with prepared vaccine and challenged with heterologous *P. multocida* serotype 4 (P-1662). Concerning to group three and four, in which chickens were vaccinated with commercial multivalent bacterin and infected with *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1622), mortality rates were 6.7% and 13.3%, respectively. In non-vaccinated groups (five and six) and challenged with *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1662), mortality rates were 73.3% and 80%, respectively. chickens of group seven (control) which is non-vaccinated and non-challenged, they show no mortalities. Results of mortality and protection rates were summarized in Table 2.

Molecular evaluation of prepared bacterin by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Results of SDS-PAGE showed that *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1662) which were grown *in-vivo* by inoculation on chicken embryonating eggs via allantoic and yolk sacs inoculation (lanes 1, 2, 3 and 4), they showed an over expressed specific bands at 35kDa and 39kDa. While *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1662) which were grown *in-vitro* by inoculation on brain heart infusion broth and agar (lanes 5, 6, 7 and 8), they did not show any specific bands. Results of SDS-PAGE are shown in Figure 2.

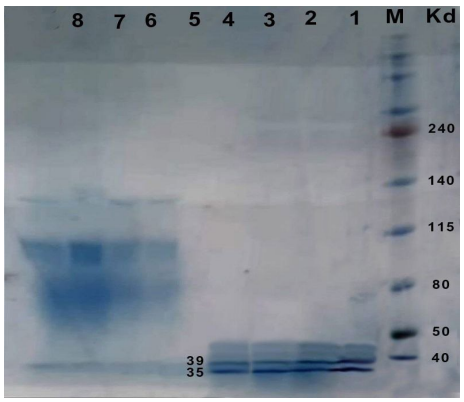

Fig. 2. SDS-PAGE of *P. multocida* grown *in-vivo* and *in-vitro*. M: pre-stained protein marker; lane 1: *P. multocida* serotype 3 (P-1059) grown *in-vivo* (allantoic sac); lane 2: *P. multocida* serotype 3 (P-1059) grown *in-vivo* (yolk sac); lane 3: *P. multocida* serotype 4 (P-1662) grown *in-vivo* (allantoic sac); lane 4: *P. multocida* serotype 4 (P-1662) grown *in-vivo* (yolk sac); lane 5 and 6: *P. multocida* serotype 3 (P-1059) grown *in-vitro* (BHI broth); lane 7 and 8: *P. multocida* serotype 4 (P-1662) grown *in-vitro* (BHI agar).

Table 1. Clinical signs and postmortem lesions of experimental infection.

Group No.	Vaccination	Infection	Clinical signs	PM lesions
1	Prepared bacterin	*P. multocida* serotype 3 (P-1059)	Moderate clinical signs	Moderate PM lesions
2	Prepared bacterin	*P. multocida* serotype 4 (P-1662)	Moderate clinical signs	Moderate PM lesions
3	Commercial vaccine	*P. multocida* serotype 3 (P-1059)	Mild clinical signs	Mild PM lesions
4	Commercial vaccine	*P. multocida* serotype 4 (P-1662)	Moderate clinical sings	Moderate PM lesions
5	Not vaccinated	*P. multocida* serotype 3 (P-1059)	Severe clinical signs	Severe PM lesions
6	Not vaccinated	*P. multocida* serotype 4 (P-1662)	Severe clinical signs	Severe PM lesions
7	Not vaccinated	No infection	No clinical signs	No PM lesions

Table 2. Mortality and protection rates in different groups of experimental infections.

Group No.	Vaccination	Infection	Mortality		Protection
			No.	%	%
1	Prepared bacterin	*P. multocida* serotype 3 (P-1059)	2/15	13.30%	86.70%
2	Prepared bacterin	*P. multocida* serotype 4 (P-1662)	3/15	20%	80%
3	Commercial vaccine	*P. multocida* serotype 3 (P-1059)	1/15	6.70%	93.30%
4	Commercial vaccine	*P. multocida* serotype 4 (P-1662)	2/15	13.30%	86.70%
5	Not vaccinated	*P. multocida* serotype 3 (P-1059)	11/15	73.30%	26.70%
6	Not vaccinated	*P. multocida* serotype 4 (P-1662)	12/15	80%	20%
7	Not vaccinated	No infection	0/15	0%	100%

Discussion

Poultry industry around the world including Egypt faces many problems, fowl cholera is one of the most important of them, so vaccination against the infection is used to control the incidence of the disease (Gergis, 1978). Living and inactivated fowl cholera vaccines had been used to prevent the disease (Glisson *et al*., 2008). Inactivated *P. multocida* bacterins are commonly used because the bacteria do not revert to be virulent and cause the disease (Hopkins and Olson, 1997).

Inactivated fowl cholera bacterin had been prepared from *P. multocida* strains that were grown *in-vitro* as follow; *P. multocida* strains was cultured on BHI broth at 37°C for 16-24 hours to obtain a dense culture containing approximately 10^8 CFU of each strain, then formalin (0.2 %) was added to the culture and mixture was re-incubated at 37°C for 24 hours for complete bacterial inactivation. Aluminum hydroxide gel (2%) was added in concentration of 20% and was mixed well with the formalinized cultures. The bacterin was standardized to contain 10^8 CFU/0.5ml dose. The quality control parameters of bacterin were tested (Borkowska-Opaka *et al*., 2000; Abd El-Ghany *et al*., 2020). In the present study, inactivated fowl cholera bacterin was prepared from *P. multocida* serotype 3 (P-1059), which was grown *in-vivo* by inoculation into chicken embryonating eggs via allantoic sac as rout of inoculation. Harvested allantoic fluids were inactivated with formalin (0.3%) and then incubated at room temperature 24 hours to allow complete inactivation of bacterial strains. Formalinized embryonic fluids equally mixed with Freund's vaccine adjuvant and the prepared bacterin was tested for quality control parameters according to OIE (2021). Our results about preparation of fowl cholera chicken embryo derived inactivated bacterin are in partial agreement with those obtained by Ibrahim *et al*. (2002), who reported that when *P. multocida* strains were grown *in-vivo* by inoculation in chicken embryo, this may lead to acquisition of new antigens which expressed into new proteins that may results in demonstration of cross protection among *P. multocida* homologous and heterologous serotypes (Ibrahim *et al*., 2002).

In the current study, results of experimental infection showed that clinical signs appeared 3 days post challenge, sudden death occurred in non-vaccinated challenged chickens. Clinical sings include depression, decreased feed and water intakes, whitish diarrhea and respiratory symptoms as cough, rales, nasal and ocular discharges, and facial edema. Clinical signs were severe in non-vaccinated infected birds and mild to moderate in vaccinated infected birds. Chickens of control group showed no clinical signs. An Egyptian study reported that clinical symptoms of *P. multocida* in vaccinated infected chickens were mild depression, off food, diarrhea, septicemic picture, and congestion of mucous membrane of buccal cavity and conjunctiva. Non-vaccinated infected chickens showed severe clinical signs, while no clinical signs appeared in the non-challenged control chickens (Abd El-Ghany *et al*., 2020). Another study recorded signs of dullness, depression, anorexia, greenish diarrhea, and difficult breathing in *P. multocida* infected non-vaccinated birds, while the vaccinated chickens did not show clinical symptoms except dullness and depression (Levy *et al*., 2013). Also, signs of fowl cholera in commercially laying hens were reported as depressed birds, anorexia, ruffled feathers, oral and nasal mucous discharge and cyanosis of comb and wattles (Mehmood *et al*., 2016).

Our results for postmortem lesions showed that, some chickens dead fleshy and in good condition, there was generalized congestion and septicemia, liver, spleen, and kidneys were affected, also there was congestion and exudates in trachea and bronchi, lungs showed edema and congestion, enteritis in intestine and pancreas was enlarged, congested and hemorrhagic. Postmortem lesions were severe in non-vaccinated infected chickens and mild to moderate in vaccinated infected chickens. Chickens of control group showed no postmortem lesions. These lesions were in accordance with a study carried out by Abd El-Ghany *et al*. (2020), who reported mild and severe postmortem lesions in *P. multocida* vaccinated-challenged and non-vaccinated-challenged control chickens, respectively. The lesions included septicemic picture, congested internal organs, enlargement of liver with sub-capsular hemorrhage, pericarditis and enlargement and congestion of spleen. Another study showed that postmortem lesions of chickens vaccinated with two doses of *P. multocida* local bacterin were congestion of heart with liver and spleen were slightly congested, however the lesions in non-vaccinated-infected chickens were congested subcutaneous blood vessels, muscles were dark and red, liver and spleen were enlarged and congested with pericarditis (Ashraf, 2000). Our results disagree with a study that reported general congestion, hemorrhages, exudates in pericardium and peritoneum, spleen and liver were enlarged with white necrotic foci on liver in chickens vaccinated with type A:1 of fowl cholera (Herath *et al*., 2010).

Concerning to mortality and protection rates of prepared fowl cholera chicken embryo inactivated derived bacterin in comparison to commercial multivalent vaccine, results were summarized in (Table 2). Results showed that prepared fowl cholera chicken embryo derived bacterin provides protection rates 86.7% and 80% against homologues *P. multocida* serotype 3 (P-1059) and heterologous *P. multocida* serotype 4 (P-1662) infections, respectively. These protection rates resemble those of commercial multivalent fowl cholera bacterin, containing *P. multocida* serotypes 1,3, 3/4 and 4, against the same infections of *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1662), it gave protection rates 93.3% and 86.7%, respectively. These results suggested that fowl cholera bacterin which is prepared from *P. multocida* grown *in-vivo* by inoculation on chicken embryonating eggs has cross protection against homologues and heterologous infections. These results disagree with a study in which double doses of prepared bacterin induced good protection rates (80-90%) against challenge with *P. multocida* of homologous challenge, but low protection rates (10-30 %) against heterologous infection (Herath *et al*., 2010). Abd El-Ghany *et al*. (2020) detected the mortality and protection rates of two locally prepared bacterins and results pointed out that mortality rates were 15% and 20% in vaccinated infected chickens with *P. multocida* type A:1 and A:3; respectively, however, in non-vaccinated infected birds, they were 90% and 80% for *P. multocida* type A:1 and A:3; respectively. In the same study, protection rates were 85% and 8 % in vaccinated infected chickens with *P. multocida* type A:1 and A:3; respectively, however, in non-vaccinated infected birds, they were 10% and 20% for *P. multocida* type A:1 and A:3; respectively. Similar results were obtained by other authors (Ashraf, 2000; Mariana and Hirst, 2000).

Concerning to molecular evaluation of prepared fowl cholera chicken embryo derived inactivated bacterin, results of SDS-PAGE showed that bacterial strains including *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1662) which were grown *in-vivo*, they showed an over-expressed specific bands 35kDa and 39kDa. *In-vitro* growing of *P. multocida* serotype 3 (P-1059) and *P. multocida* serotype 4 (P-1662) did not show any specific bands on SDS-PAGE. Our results agree with Ibrahim *et al*. (2002), who reported that *P. multocida* serotypes grown *in-vivo* demonstrated different electrophoretic mobilities than *P. multocida* grown *in-vitro*. The antigen 39kDa appeared very dense, suggesting an alteration in protein structure of the *P. multocida* strains . It was stated that 39kDa is a dominant protein in *P. multocida* grown *in-vivo* (Rimler, 1994; Ibrahim *et al*., 2002). It was suggested that 39kDa may play a role in antigenic cross protection among *P. multocida* serotypes. Another study was carried out by Poolperm *et al*., (2018), in which they evaluate an internasal recombinant fowl cholera vaccine in ducks, results of SDS-PAGE showed expression of specific band at approximately 39kDa in recombinant *P. multocida* serotype 1 (X73).

Conclusion

Fowl cholera inactivated bacterin is usually prepared from multivalent *in-vitro* growth of *P. multocida* serotypes, but in the present study, it was possible to prepare fowl cholera bacterin by *in-vivo* growing of *P. multocida* serotype by inoculation into chicken embryonating eggs. Experimental infection was carried out to evaluate the prepared bacterin in comparison to commercial multivalent vaccine, results revealed that prepared fowl cholera chicken embryo derived inactivated bacterin is effective against infection of homologous and heterologous *P. multocida* serotypes with low mortality and good protection rates. Molecular evaluation of the prepared fowl cholera chicken embryo derived inactivated bacterin using SDS-PAGE results in expression of new specific proteins which may play a role in cross protection among different serotypes of *P. multocida*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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