Protective efficacy of plasmid DNA vaccine coding for OmpH gene of Pasteurella multocida


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Abstract:

Fowl cholera is one of the important problems in poultry industry. Fowl cholera is caused by P. multocida. This disease has been poorly controlled and researchers are still looking for an effective vaccine. In the present study, P. multocida major outer protein gene (ompH) reported to be strong immunogens. The gene (ompH) encoding OmpH protein had been amplified by PCR technique from the whole genome of P. multocida CU strains and expressed in E. coli after cloned between BamHI and SalI sites of pBK-CMV phagemid vector. In SDS-PAGE analysis, the OmpH protein contains a major band with a molecular mass of about 37 kDa. Comparing this with the Western blot assay revealed that there is the faint band was able to react to the P. multocida positive chicken serum antibody. Chicken (6-8 weeks) were vaccinated twice 3weeks intervals by inoculation of 100 μg DNA. Another group of chicken was vaccinated with P. multocida bacterins. The results of IHA and ELISA test, revealed that the DNA vaccine gave pronounced protection. All vaccines reach to maximum mean titre at 3rd week post vaccination and at 2nd week post boosting.

The protection percentages after challenge were 87.5 and 90% in case of DNA and inactivated vaccine respectively. Finally, it was found plasmids encoding ompH gene of P. multocida induce antibodies against P. multocida and gave a good protection.

Key words: Pasteurella, vaccine, DNA, cloning.

Introduction:

Fowl cholera is an acute or chronic infectious disease of domestic and feral birds. It had been recognized in avian species for many years (Kim and Nagaraja, 1990). In Egypt, fowl cholera is one of the important problems in poultry industry (Gergis, 1987) and considers being the most costly bacterial respiratory disease in poultry. The disease is caused by P. multocida which belongs to the family Pasteurellaceae.
The current study aimed to produce the plasmid DNA vaccine encoding the oomph gene of *P. multocida* and evaluate its immunogenic and protective efficacy in chicken

**Materials and Methods**

**Strains**

1- **Vaccinal strain of *P. multocida***

A virulent strain of *P. multocida*, Clemson University (CU) strain was obtained from the Schering-Plough Animal Health Company. It was passed in rabbits to increase its virulence and to keep the organism actively capsulated. It was used for preparation of DNA vaccine

2- **Virulent standard strains of *P. multocida***

Serotypes 3 (A:8), 1 (A:5), 4 (A:9) and D2 were obtained from the Strain Bank Dept., Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Cairo. They were also passed in rabbits to maintain their virulence for the challenge test.

**Vaccines**

Two types of vaccines were used in this study. The DNA vaccine prepared in this study and the killed *P. multocida* vaccine (IZOvac Fc emulsified inactivated vaccine against Fowl Cholera manufactured by IZO.S.P.A via A. Bianchi 9 - Brescia Italy).

**Sampling**

Blood samples were collected through heart puncture and jugular vein from chicks pre vaccinated and weekly post vaccination. The sera were separated by centrifugation at 2500 rpm after 10 min. and kept in dry sterile screw capped tubes at – 20°C till used.

**Extraction of genomic DNA of *P. multocida* : (Sambrook et al., 1989)**

A single bacterial colony from a pure culture of *P. multocida* strains was inoculated in trypticase soy broth with 1.25% tryptose and incubated overnight at 37°C, centrifuged at 4000 rpm for 5 minutes at 4°C. the pellet was washed with TE buffer, digested for 2 hours at 37°C with 150 µl of lysozyme (final concentration...
10 mg/ml) then 40 μl of 10% SDS solution were added and mixed for 1 minute. 60 μl of proteinase K (20 mg/ml) solution were added and incubated at 65°C for 30 minutes DNA was extracted by addition of 800 μl of buffer saturated phenol and then centrifuged at 14000 rpm for 10 minutes. The aqueous phase was re-extracted with equal volume of phenol: chloroform: isoamyl alcohol and then with chloroform: isoamyl alcohol and centrifuged as before. The DNA in the upper aqueous phase was precipitated with double volume of ice cool absolute ethanol. The precipitated DNA was centrifuged at 14000 for 30 minutes at 4°C, washed in 1 ml of 70% cooled ethanol and centrifuged as before. Finally the DNA was dried in a vacuum dissector for 5 minutes and suspended in 50 μl of TE buffer. Purification of the genomic DNA was done using Wizard DNA clean up system (Promega) according to the manufacture instruction.

**PCR amplification**

PCR was performed in 50-μl reaction mixtures containing 50 mM KCl, 10mM Tris-HCl (pH8.8), 3 mM MgCl₂, 200 mM (each) deoxynucleoside triphosphate, 10μl Q solution (Qiagene) and 1 U of thermostable taq DNA polymerase, 500ng of purified DNA and 50 pmol of each oligonucleotide primer. The primers used in this study were corresponding to the position of (−3 to 19) forward by pass the native RBS (Ribosomal binding site), and position (150-138) downstream the stopping codon (Townsend et al., 1998), to amplify the full length omph gene. To this sequence a recognition site for salI was added to the forword primer and BamHI in the reverse one to facilitate the cloning in the phagemid vector. Thermal cycling was performed using T gradient thermalcycler (Biometra, Germany), the parameters for amplification were denaturation at 94 °C for 2 min for one cycle and then 40 cycles at 94°C for 1 min, 55 °C for 1 min, and 72°C for 1 min. A final extension at 72 °C for 10 min was also included.

**Cloning Cloning of a 1,000-bp fragment encoding omph gene of P. multocida**

Cloning procedures were done according to Sambrook et al., (1989). briefly, the purified DNA of *P. multocida* (20 μg) was digested with both SalI (to prepare the 5' end) and BamHI (to
prepare the 3' end), and a fragment of about 1,000 bp was cut and extracted from the 1% low melting agarose gel by wizard PCR purification kit (Promega). Ligation was made in pBK-CMV phagemid Stratagene Co., USA, (Cat. No. 212209), previously digested with SalI and BamHI, using the T4 DNA ligase (Roche). Transformation was made in competent XL1 blue cells (Gibco BRL) by Heat shock method. Cultures were incubated overnight on tetracycline (12 μg/ml), kanamycin (50 μg/ml) containing agar plates. Addition of isopropyl-b-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-Gal) allowed us to distinguish between colonies containing the insert (white) and colonies without the insert (blue). Each positive individual colony was picked up and inoculated in 10 ml of antibiotic containing LB broth and incubated overnight at 37°C.

**RFLP analysis of the recombinant phagemid vector:**
(Sambrook et al., 1989)

To verify the successful cloning (in the sense orientation) of the omph gene in the phagemid vector, overnight culture of the white colonies were subjected to preparation of the recombinant phagemid (mini-prep) using high pure plasmid isolated kit (Roche Molecular Biochemicals, Germany) and the recombinant phagemid was digested with both BamHI and SalI, and fractionated on 1% agarose.

**Rapid screening of small expression cultures:**

Ten milliliter of LB-broth containing antibiotics were inoculated with 100μl of overnight culture and incubated at 37°C/2 hours with shaking till the OD600 is 0.5-0.7. and the expression was then induced by adding 1 mM IPTG and 0.1 mM PMSF and incubated at 37°C/4 hours. Samples were then taken from both induced and non-induced culture and subjected for SS-PAGE (Sambrook et al., 1989).

**Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

Samples of induced and non-induced culture were resolved on discontinuous buffer system composed of 12% (w/v) acrylamide
incubator at 37°C until hatching. The hatched chicks were reared in completely sterile isolators.

1- DNA vaccine

The chickens injected I/M with either 100µg of DNA vaccine in PBS (group 1) or emulsified in incomplete Freund’s adjuvant (group 2), and boosted 3 weeks later.

2- Killed *P. multocida* vaccine

Another group of SPF chickens were inoculated with killed *P. multocida* vaccine (group 3)

3- control group

A group of SPF chickens kept in a separate place without any treatment to serve as negative control

Evaluating the immune response of the vaccinated chickens:

A- ELISA technology

Nunc microtiter plates were charged with 100 µl of sonicated antigen diluted 1:10 in carbonate bicarbonate buffer. After incubation at 4 °C for 24h the remaining active sites were blocked with 3%BSA. Serum samples were diluted at 1/5 in PBS and each well received 100µl. A standard positive and negative serum samples (P.M coated plated Vitro Diagnostikum Gebrauchs information entnehmen cat # 04-01172-03) were included in this test to facilitate the calculation of S/P ratio. Anti-chicken peroxidase labeled secondary antibodies was used at dilution of 1/10,000. OPD was used as a substrate and the O.D was read at 450 Å. The results were calculated according to the formula described by Briggs and Skeels (1984).

\[
\text{Log}_{10} \text{titer} = 1.08 (\log_{10} \text{S/P}) + 3.82
\]

Where:

\[
\frac{S/G \text{ Ratio}}{= \frac{\text{SampleMean} - \text{NegativeControl}}{\text{PositiveControl} - \text{NegativeControl}}}
\]

B- Indirect haemagglutination test
It was done according to Carter and Rappay, (1962) using the lipopolysaccharide (LPS) capsular antigen of \textit{P. multocida} for sensitizing the formalized RBCs used in the test.

\textbf{C-Challenge test}

Three weeks after the vaccination, the chickens were challenged I/M by 0.1 ml of virulent \textit{P. multocida} (A:8)

\textbf{Results}

\textbf{Results of PCR amplification and cloning of omph gene}

The \textit{omph} gene was amplified from the genomic DNA of \textit{P. multocida} CU strain by PCR using Taq polymerase with high validity, when DNA concentration was 500ng/reaction a clear visible band migrate at 1000 bp was visualized under the UV illumination Fig 1.

After heat shock transformation of \textit{E. coli} XL1 blue competent cells with the constructed plasmid, over than 50 white colonies /plate were grown after overnight incubation at 37 \textdegree C. Miniprep analysis and RFLP Fig (2) showed that over 80% of the colonies were positive (expressing the re OMP H antigen).

Digestion of the recombinant plasmid by using \textit{BamHI} and \textit{SalI} restriction enzyme, are shown in Fig 2 and electrophoretic analysis revealed that the plasmid fragment was detected without inserts of PCR product (4.5 kbp), whereas the other fragments were around (5.5 kbp) representing the insert as a concerning the DNA marker.

\textbf{Results of induction of expression protein by using SDS-PAGE and Western blot analysis}

SDS-PAGE of the protein profiles of both the recombinant cells and the negative control competent cells Fig 3 revealed marked differences in the electrophoretic profile at the molecular weight level of 37 KDa. As shown the 37 kDa protein was present only in the recombinant cell. Western blot analysis for the recombinant protein from the recombinant cells using specific \textit{P. multocida} antisera demonstrated that the 37 KDa protein was present in the recombinant cells (Fig . 4).
Fig 1. Show the PCR product of ompIIF gene of CU (Clemson University) strain using specific primer with sticky end.

Lane (1): Kb DNA ladder (250-12000 base pair).
Lane (2): PCR product of ompIIF gene of the CU strain.
Lane (3): Whole purified genomic DNA of CU strain.
Lane (4): Whole genomic DNA of CU strain without Purification

Fig 2. Show the results of restriction enzyme digestion of unrecombinant plasmid and recombinant plasmid with ompIIF gene insert.

Lane (1,6): Kb DNA ladder (250-12000 base pair).
Lane (2): Digested unrecombinant plasmid only (4518bp).
Lane (3): Digested recombinant plasmid with ompIIF gene.
Lane (4,5): Recombinant plasmid with ompIIF gene insert (5518).
Results of maxiprep.

For DNA vaccination of chickens, a large scale plasmid preparation "maxiprep" was clone. From the white colonies shows (+ve) orientation of gene in plasmid for the miniprep analysis. The plasmid was then purified using Wizard plasmid purification kit (Promega). The concentration of plasmid before and after purification were 10 μg/μl.

Results of the experimental study to evaluate the prepared DNA vaccine

The passive IHA test, (Table 1 and Fig. 5) showed marked increase in the IHA titre (1810) till it reached to 1024 at 3rd week among chicken vaccinated with the prepared DNA vaccine without adjuvant. After boostering a slight decrease in the titre (608) was recorded, but it increase gradually till reached (861). A similar results were recorded among the chicken group received DNA vaccine with Freund’s adjuvant, but differ in the amplification, the titre was (279.2) at 1st week post vaccination and increased till it reached to 689.1 at 3rd week. The titre decreased again at the boostering to 380.4 and increase gradually till 645 at 4th week. One week post challenge, the titres were 512.0 and 406 among the group vaccinated with the DNA vaccine without adjuvant and DNA with Freund’s respectively.

![Fig 3.SDS-PAGE analysis of induction of expression Omph1 protein show the presence of recombinant Omph1 protein into band at 37 KD.](image)

On the other hand, the chicken vaccinated with *P. multocida* killed vaccine had antibodies titre 724.1 after 3rd week post vaccination. Boosting resulted in slight decline in the titre 406 which again increase once more to 1351.176 at 5th week. Challenge resulted in slight decrease in titre.

ELISA was used for measuring the humoral immune response of chicken against DNA vaccine of *P. multocida*. Evaluation of the antibody titers in sera of chickens vaccinated with the DNA *P. multocida* vaccine (group 1) using ELISA from (Table 2 and Fig. 6), it is clearly that the inoculation of DNA vaccine by I/P and I/M routes caused high activity in antibody titers and it reached to 8155.2 at 3 weeks post vaccination. Meanwhile, the titer of antibodies was 6821.9 among chicken group vaccinated by DNA *P. multocida* vaccine (group 2) with Freund’s adjuvant during the same time interval. Also, a rapid rise of antibody titer was observed after vaccination with a booster dose in group 1 and group 2 as the titer of antibodies reached 7285.3 and 5196.6 respectively, at 2 weeks post boosting dose after challenge antibody titer declined to 4945.5 and 4754.18 in the vaccinated chicken sera in group (1) and group (2) respectively. On the other hand, the level of antibody titers in chicken vaccinated with killed vaccine reached to 11955.5 at 3 weeks post vaccination. While, the maximum level of antibody titers observed at 2 weeks post challenge (14217.3).

The immunity of the vaccinated chicken in group 1, 2 and 3 as well as the control unvaccinated chicken in group (4) were challenged by a virulent strain of *Pasteurella multocida* 8:A serotype (3) through I/M route, the LD<sub>50</sub> of *P. multocida* challenging strain was (10<sup>6</sup>). The results was tabulated and shown in Table 3, indicated that the protection rate was 87.5 % in group 1 and 2 (each), 93% in group 3, while all chickens in group 4 died.

**Discussion**

*Pasteurella multocida* (pm), a widespread Gram-negative eubacterium, alone or in association with other pathogens, is responsible for sever diseases in mammals (including man) and birds (Chanter and Rutter, 1981) such as fowl cholera in chickens.
and turkeys, atrophic rhinitis in swine and hemorrhagic septicemia in cattle and buffaloes.

**TABLE 1. Results of the humoral immune response of the vaccinated chicken using indirect haemagglutination test (IHA).**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>The mean antibody titres of chicken vaccinated with:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>P. multocida</em></td>
<td><em>P. multocida</em></td>
<td><em>P. multocida</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DNA vaccine</td>
<td>DNA vaccine</td>
<td>killed vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>with Complete</td>
<td>with Complete</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Freund's adjuvant</td>
<td>Freund's adjuvant</td>
<td></td>
</tr>
<tr>
<td>Pre vaccination</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>1st week PV</td>
<td>181.0</td>
<td>279.2</td>
<td>161.3</td>
<td></td>
</tr>
<tr>
<td>2nd week PV</td>
<td>388.0</td>
<td>438.9</td>
<td>463.73</td>
<td></td>
</tr>
<tr>
<td>3rd week PV</td>
<td>1024.0</td>
<td>689.1</td>
<td>1176.267</td>
<td></td>
</tr>
<tr>
<td>1st week PB</td>
<td>608.8</td>
<td>380.4</td>
<td>724.1</td>
<td></td>
</tr>
<tr>
<td>2nd week PB</td>
<td>861.1</td>
<td>645.1</td>
<td>1351.176</td>
<td></td>
</tr>
<tr>
<td>1st week post challenge</td>
<td>512.0</td>
<td>406.4</td>
<td>776.0</td>
<td></td>
</tr>
<tr>
<td>2nd week post challenge</td>
<td>552.0</td>
<td>512.0</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>PV: Post vaccination</td>
<td>PB: Post boosting</td>
<td>ND: Not done</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
One of the key steps in the process is the identification of the gene(s) to be cloned, these genes are frequently associated with products involved in virulence or are major surface antigens. The outer membrane of gram-negative bacteria contains lipopolysaccharides, several minor proteins and a limited number of major proteins present in very high copy number (Hancock, 1991). One of the major outer membrane proteins (MOMP s) is called porins (Vasfi Marandi and Mittal, 1996). OmpH is a protein involve in the synthesis of LPS, or in the exportation of macromolecules through the bacterial envelope as recently demonstrated (Thome and Muller, 1991). Protein H or porin H is the major outer membrane protein in the envelope of *Pasteurella multocida*, so this protein has chosen to prepare a recombinant vaccine.
Fig 5. Results of IHA test of chicken vaccinated with *P. multocida* DNA vaccines either alone or with Freund’s adjuvant and *P. multocida* killed.

WPV: week post vaccination.
WPB: week post booster ing.
WPC: week post challenge.

Firstly *Pasteurella multocida* strains (CU) was subjected to extract the genomic DNA using traditional phenol chloroform method. Due to the tense cell wall of gram negative bacteria, enzymatic treatment of the strains were applied before the chemical extraction that include treatment of proteinase K and lysozymes to weaken the cell wall before chemical extraction of genomic DNA (Sambrook *et al.*, 1989 and Capron and Scott, 1991). Because the genomic DNA will be used for application of *ompH* gene by PCR and will subsequently cloned in mammalian expression vector, high pure DNA must be used, so the genomic DNA was further purified using Wizard genomic DNA purification kit, and this resulted in increase the purity of extracted DNA.

The *ompH* gene was amplified from genomic DNA of *Pasteurella multocida* strains by using PCR. The nucleotide sequence of *ompH* gene was first described by Luo *et al.* (1997). The gene was isolated from *Pasteurella multocida* X-73 strain and contain the signal region which corresponding to position of
1-63 base pair, with the presence of inverse repeat 15 bp downstream the stopping codon, and multiple sequence alignment of OmpH amino acid sequence of different serotype revealed high homology (72.3% overall identity) with variation of amino acid composition and sequence length in some regions. Major variation will confined to two discrete regions, amino acids 60-80, and 200-220 (Luo et al., 1997).

**TABLE 3. Results of challenge test**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>Total No. of chicken</th>
<th>No. of died chicken</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Chicken vaccinated with DNA PMV</td>
<td>16</td>
<td>2</td>
<td>87.5</td>
</tr>
<tr>
<td>G2</td>
<td>Chicken vaccinated with DNA PMV with Freund’s adjuvant</td>
<td>16</td>
<td>2</td>
<td>87.5</td>
</tr>
<tr>
<td>G3</td>
<td>Chicken vaccinated with killed Fowl cholera vaccine</td>
<td>20</td>
<td>2</td>
<td>90.0</td>
</tr>
<tr>
<td>G4</td>
<td>Control group</td>
<td>20</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

PMV: *Pasteurella multocida* Vaccine.

For cloning and expression of *ompH* gene, mammalian cloning vector was constructed and used for transformation of *E. coli* host strain XL1 Blue, this plasmid (Phagemid) which is prokaryotic and eukaryotic expression. CMV promoter (1895-1306) eukaryotic expression is driven by the CMV immediate early promoter. The cloning sites located with the MCS for either prokaryotic or eukaryotic expression. Cloning between Nhe I site (5’) and MCS (3’) allows high-level eukaryotic expression when the insert contains an ATG and Kozak sequence. After ligation and transformation of *E. coli* host strain by heat shock method, a high yield of transformed was obtained which were used in subsequent steps. Analysis of positive transformant was done by miniprep and SDS-PAGE electrophoresis of induced overnight culture. First mini-prep analysis of white (transformed colonies) shows a band migrated at 5.5 kbp which is equal to the theoretical length of the recombinant plasmid (the native plasmid form 4.5
kbp and omph gene form 1 kbp), and the RFLP analysis of this recombinant plasmid revealed two bands at 4.5 kbp and 1 kbp on using Sal I and Bam HI which have recognition sequence up- and down-stream of omph gene, respectively, which corresponding to the linearized native plasmid and 2nd band is the insert.

![Graph showing ELISA titers](image)

**Fig 6.** The mean ELISA titer of chicken vaccinated with P. multocida DNA vaccine either alone or with Freund’s adjuvant and P. multocida killed vaccine.

WPV: week post vaccination.
WPB: week post boosting.
WPC: week post challenge.

The ligation products were transformed into competent cells of XL1 Blue E. coli. Since the intent in vaccine development is to isolate and use highly immunogenic antigens, the simplest way to identify the correct recombinant clones is by using an immunos assay. Ideally, the screening of recombinant can be accomplished by blotting the colonies into nitrocellulose filter and detecting with enzyme-labeled antibodies against the native antigen.

In order to evaluate whither, the insert is cloned in sense orientation (in ORF with the plasmid’s Ribosomal binding site), the white colonies were selected and transformed to LB media with antibiotic and overnight incubation has been done, from which induction of IPTG is undertaken and aliquots of induce and

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non-induce culture were analyzed with SDS-PAGE and Western blot.

SDS was applied to identify the presence of recombinant protein in the recombinant cells after cloning. As shown in Fig (3), the 37 kDa protein was present only in the recombinant cell. Also, using specific *Pasteurella multocida* antisera demonstrated the 37 kDa protein (Fig 4). The molecular masses of denaturated omp range between 34 and 42 kDa depending on the serotype and the electrophoretic system used for analysis (Hancock and Benz, 1986 and Chevalier et al., 1993). Furthermore, Luo et al., 1997 recorded that the fully denaturated monomer of OmpH has a molecular mass of approximately 37 kDa. High molecular weight band also seen which may represent dimmers or polymers of the protein, although Luo et al., 1997 found the same molecular weight band on using in boiled samples but usually the recombinant protein (specially when induction is done at 37°C not at 32°C result in over expression of the protein with minimum post transcriptional modification resulting in very stable aggregate formation which may resist boiling and needs more time (over 10 min.) or even chemical refolding (30% polyethylene glycol) (Wiker et al., 1998 and Bowden and Georgion et al., 1990). In previous studies (Chevalier et al., 1993 and Lubke et al., 1994) found that the native confirmation of porin H is homotrimer stable in SDS at room temperature and dissociated into monomers upon boiling resulting in denaturing monomers ranges between 34-42 kDa depending on serotype and electrophoretic system used for analysis. Western blot analysis revealed strong band at 37kDa and another minor band below it (34 kDa) and another intense band at a molecular weight over 97 which represents the monomeric and the aggregate forms of the recombinant protein of *ompH* gene.

An attempt was carried out to evaluate the prepared DNA vaccine. A group of SPF chickens were injected I/M by 100 µg/ml of the prepared vaccine and boostering after 3 weeks post vaccination. DNA injected intramuscularly had been found to distribute throughout muscle, with the DNA diffusing through the extracellular matrix, crossing, the exact mechanisms involved in the uptake of DNA by individual muscle cells are unknown, but uptake by some type of cell membrane transporter has been
proposed (Wolff et al., 1992). The transport of plasmid DNA into the cell nucleus appears to occur through the nuclear pore by a process common to other large Karyophilic macromolecules (Dowty et al., 1995).

The humoral immune response using IHA test, as shown in Table 1 and Fig. 5. It was found that the titre increased rapidly and reached the maximum titre at 3\textsuperscript{rd} post vaccination. Then decline after boostering and again increase once more till the 2\textsuperscript{nd} week post boostering. It was noticed that using of adjuvant did not affect the overall result yet the antibody titre was slightly lower when the adjuvant was omitted and that because, the water in oil emulsion may not allow maximum release of the plasmid with subsequent minimum expression of the protein which encounter the immune system, after challenge the titre was rapidly decline one week post challenge which indicate the consumption of haemagglutinating antibodies in the protection process. As shown in Table 1 and Fig. 5, that the titre measured by passive haemagglutination was 279.2, 181.0 and 161.3 at 1\textsuperscript{st} week respectively and increase till reach to 689.1, 1024.0 and 1176.267 at 3\textsuperscript{rd} week. After boostering was slight decrease in the titre 380.4, 608 and 724.1, respectively which again increase gradually till reached to 645.1, 861.1 and 1351.176.

It worth notice that, the antibody level decline after boostering, then increases two weeks later, this is because the partial neutralization of circulating antibodies with the newly expressed protein yet the overall humoral response still high (9243). Measurement of neutralizing antibodies using ELISA (Table 2 and Fig 6) revealed near the same pattern of IHA test which may indicate that, the DNA encoding vaccine produce hemagglutinating and neutralizing antibody nearly the same.

The most effective method to test the protective immunity is the challenge test. In the undergoing experiment the challenge test is DNA vaccine groups gave 87.5% protection, while using killed Pasteurella multocida vaccine give 93.75% protection, this result may be explained on the bases that killed vaccine gave chance to the immune system to deal with the whole microorganism resulting in production of antibodies against a wide panel of antigens rather than dealing with one antigen.
To evaluate the protection efficiency of the prepared DNA, the vaccinated chicken groups were challenged by a virulent *P. multocida* A: 8 strain intramuscularly. As shown in Table 3, the protection rates were 87.5% and 93.75% among the chicken vaccinated with DNA *Pasteurella multocida* vaccine and killed *Pasteurella multocida* vaccine respectively, (Luo et al., 1997) recorded that the purified OmpH induced 100% protection but the recombinant OmpH induce little protection.

In conclusion, although the killed vaccine gave more protection yet, the DNA vaccine proofed to be effective in the protection against fowl cholera vaccine.

References


