EFFICACY OF FORMALIN KILLED POULTRY cholera vaccine in EXPERIMENTALLY IMMUNIZED FAYOUNM chickens


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ABSTRACT

Efficacy of experimentally prepared formalin killed fowl cholera vaccine in Fayounm turkeys through six doses of vaccination was determined during the period from April 2002 to March 2003. Pasteurella multocida (PM 38) serotype 1 (X-73) was employed for vaccine preparation and antibody titre of the chicken sera were assessed by passive haemagglutination (PHA) test. Vaccination was done either experimentally or naturally. Each of the experimental chickens was challenged with a standard inoculum of P. multocida of 3.3 x 10^6 CFU/ml per bird intranasally. The 100% vaccinated chickens protected against P. multocida infection but all 100% unvaccinated control birds died within 2 hours of challenge. Intranasal (both primary and booster) dose of vaccinations was found superior and more effective than subcutaneous route of administration. The higher PHA antibody titre was recorded with intranasal (0.223±0.003) dose than subcutaneously (1.16±0.031) dose vaccinated group of birds. The results revealed the fact that intranasal route followed by subcutaneous inoculation could be done for vaccination against fowl cholera in chickens.

Key words: Efficacy, fowl cholera vaccine, formalin killed, Fayounm chicken

INTRODUCTION

Fowl cholera is one of the most important contagious bacterial diseases of poultry caused by Pasteurella multocida. It occurs sporadically or occasionally in most countries of the world including Bangladesh. It causes mortality about 25 to 50% in chickens of Bangladesh (Choudhury et al., 1995). Vaccination as a means of controlling infectious diseases of animals and birds is a universal approach. Both humoral immunity (HI) and cell mediated immunity (CMI) are considered to be of primary importance in the prevention of animals and birds against infectious diseases (Collins, 1977; Mondal et al., 1988). The immune responses (Mondal et al., 1988; Choudhury et al., 1990) efficacy of oral type bivalent vaccine (Choudhury et al., 1987) and oral precipitated (Khan et al., 1994; Islam et al., 2004) and comparative efficacy of different fowl cholera vaccines (Choudhury et al., 1988) have been evaluated under local conditions (Samad, 2001). However, a nardus titre of fowl cholera vaccine per field dose of mucosal is important for obtaining dependable immunity against the disease. This paper describes the efficacy of a formalin killed fowl cholera vaccine in Fayounm breed of chickens with their antibody responses.

MATERIALS AND METHODS

Ten weeks old (n=25) Fayounm chickens used for this study were purchased from the BAU Poulay Farm, Mymensingh on 10th April 2002. Both birds were maintained in the poultry experimental house of the Department of Microbiology and Hygiene during the period from 10th April 2002 to 15th March 2003. Pasteurella multocida (PM 38) serotype 1 (X-73) was obtained from the laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh, which was used for the preparation of formalin killed fowl cholera vaccine as well as challenge virulent organisms. These birds were divided into five experimental groups. (A, B, C, D & E) each consisting of 5 chickens which were maintained in separate cages. Each of the birds of group A, B, C and D was vaccinated with 1.0 ml of experimentally prepared formalin killed vaccine, through IM, SC and IM route respectively. Booster vaccination was done in group A, B, C and D with 1.0 ml of same vaccine through IM, SC and IM route respectively. Birds of group E served as unvaccinated control throughout the experimental period.

The immune response was studied by using growth inhibition test (GIT). Passive haemagglutination assay (PHA) and protection test to determine the presence of antibody against P. multocida in the sera of chickens immunized with formalin killed fowl cholera vaccine. GIT and PHA were conducted according to the procedure described by Tripathy et al. (1970; Riddique et al. 1997) and Islam et al. (2004).

The protection test was conducted on both vaccinated and unvaccinated groups of chickens with individual dose rate of 1.0 ml of P. multocida bacteria (3.3 x 10^6 CFU/ml) through intramuscular route is described by Choudhury et al. (1983), Khan et al. (1994) and Islam et al. (2006). The chickens were observed for one month in every 2 hours interval.

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## Efficacy of fowl cholera vaccine in Payooua chickens

### RESULTS AND DISCUSSION
The vaccine treated culture of virulent *P. multocida* was inoculated to blood agar and nutrient agar plates. No growth of bacteria on the plates after 24 hours incubation at 37°C which indicated positive GIT for vaccinated chickens, but growth was observed in case of control sample that indicated negative GIT for unvaccinated chickens (Table 1).

### Table 1. Growth inhibition test (GIT), antibody response and survival ratio of chickens immunized with formalin killed fowl cholera vaccine

<table>
<thead>
<tr>
<th>Group</th>
<th>No. birds used</th>
<th>Route of vaccination</th>
<th>Pre-vaccination Ab titre</th>
<th>Post-vaccination after 2 weeks</th>
<th>Survival ratio</th>
<th>No. (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Primary Booster</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>A</td>
<td>5</td>
<td>IM</td>
<td>4</td>
<td><strong>222.86 ± 25.60</strong></td>
<td>445.72 ± 51.20</td>
<td>5 (100)</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>IM</td>
<td>4</td>
<td><strong>168.89 ± 31.35</strong></td>
<td>388.02 ± 62.70</td>
<td>5 (100)</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>SC</td>
<td>4</td>
<td><strong>111.43 ± 12.80</strong></td>
<td>256.00 ± 60.00</td>
<td>5 (100)</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>SC</td>
<td>4</td>
<td><strong>128.00 ± 20.00</strong></td>
<td>337.79 ± 62.70</td>
<td>5 (100)</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>IM</td>
<td>4</td>
<td><strong>27.86 ± 2.30</strong></td>
<td>128.00 ± 63.20</td>
<td>5 (100)</td>
</tr>
</tbody>
</table>

IM = Intramuscular route, SC = Subcutaneous route, + = Unable to grow in culture media, - = Able to grow in culture media, *Mean ± SE, **Significant at p < 0.01.

The mean antibody titers of primary vaccination, booster vaccination and challenge exposure were 32.0 ± 0.0, 222.86 ± 25.6 and 445.72 ± 51.2 respectively, when the chickens of group A vaccinated through IM route in both primary and booster vaccination (Table 1). Similarly the mean antibody titers of group B were 32 ± 0.0, 168.89 ± 31.35, and 388.02 ± 62.70 after primary, booster and challenge exposure through IM and SC respectively. The serum mean antibody titers of chickens of group C were 27.86 ± 2.3, 111.43 ± 12.80 and 256.0 ± 0.0 after primary vaccination, booster vaccination and challenge exposure through SC respectively. In the group D, chickens were vaccinated through SC at primary vaccination followed by IM route at booster vaccination. The post-vaccination serum mean antibody titers of group B after primary vaccination, booster vaccination and challenge exposure were 27.86 ± 2.30, 128 ± 0.0 and 337.79 ± 62.70 respectively (Table 1).

The mean antibody titers of different routes of vaccination indicated that IM route of vaccination induced better results in respect of protection against experimental challenge infection and higher antibody titre and SC route of vaccination. All the four groups of vaccinated chickens induced significantly higher antibody titre in comparison with their pre-vaccination antibody titre. The findings of this experiment partly correlated with the results of Leontchuk and Tsimokh (1976) who reported that the immunogenicity of the vaccine depended on the method of vaccination and IM route gave stronger and long lasting immunity than SC route. The chickens received booster dose of vaccine induced significantly (p < 0.01) higher antibody titre than the chickens of primary vaccinated groups. The titre becomes peak after two weeks of challenge exposure (Table 1). Wu et al. (1986) observed that two inoculations provided better immunity than a single inoculation. The administration of booster dose of same vaccine induced a high level of antibody and protective immunity with no adverse reactions has been reported by Schlitt et al. (1987) and Choudhury et al. (1987). The challenge dose used for protection test was 3.8 x 10^6 CFU/ml of *P. multocida*. Chickens of all the four vaccinated group were protected against virulent *P. multocida* challenge. But all the unvaccinated control chickens died within 72 hours after challenge which has shown in Table 1.

Therefore, it may be suggested that to prevent and to reduce the occurrence of fowl cholera, the formalin killed fowl cholera vaccine prepared with highly antigenic strain of *P. multocida* should be used to provide better protection against the epidemic of fowl cholera in poultry and it is also advised to practice IM route in both primary and secondary vaccination. However, further study with large number of chickens to determine the efficacy of routes of vaccination is necessary to conclude about the present study.

### REFERENCES

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