Determination of Humoral Immune Response in Chickens Against Formalin-Inactivated Alum-Precipitated Fowl Cholera Vaccine

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Abstract

We measured the humoral immune response in chickens against a formalin-inactivated alum-precipitated fowl cholera vaccine. The vaccine was administered in 12 weeks old chickens, $5 \times 10^7$ CFU/ml/chicken intramuscularly. Booster dose was given with similar dose and route at 15, 30 and 45 days intervals in groups A, B and C, respectively, after primary vaccination. The group D served as unvaccinated control. Pre-vaccination sera were collected from all the groups of birds. Sera of the immunized and control birds were collected at 15, 30, 60, 90, 120, 150 and 180 days post vaccination (DPV). The degree of immunity produced in each group of birds following primary and secondary vaccination were measured by determining their serum antibody level using passive Haemagglutination (PHA) test. Sera sample possessing higher PHA titers after 15 days of primary vaccination manifested a declining tendency at 30 DPV and rapidly fell down at 60 DPV. Boostering of vaccination at this stage elucidated a rapid increase of PHA titers ranging from 128 to 256 at 30 DPV and declined gradually from 60 DPV and reached a titer of approximately 8 to 4 at 180 DPV. Challenge infection was conducted with five randomly selected vaccinates of all the groups of birds along with unvaccinated controls after 15 days of secondary vaccination. The fowl cholera vaccination conferred 100% protection while all the unvaccinated control birds succumbed to such infection.

Keywords

Chickens, Humoral Immune, Fowl Cholera Vaccine

1. Introduction

Fowl cholera (FC) is a highly contagious disease which is caused by *Pasteurella multocida* and has been recognized as an important disease in poultry for more than 200 years (Kwon and Kang, 2003; Glisson et al., 2008). It causes devastating economic losses to the poultry industry through death, weight loss and condemnations of carcasses worldwide (Aye et al., 2001; Glisson et al., 2008). Outbreaks of FC mostly occur in chickens, turkeys, ducks, geese, quails and Japanese green pheasants. However, the disease affects other types of poultry also, such as game birds reared in captivity, companion birds, zoo birds and wild birds (Sawada et al., 1999). FC is commonly found in mature chickens over 16 weeks of age but rarely occurs in young chickens of less than 8 weeks of age (Petersen et al., 2001; Glisson et al., 2008). The disease is seen more frequently in layers than in broilers because of age factors (Sander and Glisson, 1989).

Fowl cholera occurs sporadically or enzootically as peracute, acute or chronic form all over the world (Takai et al., 1994; Glisson et al., 2008) including Bangladesh (Choudhury et al., 1985; Baki et al., 1991). Signs of infection in acute FC are often present for only a few hours before death that includes fever, anorexia, ruffled feathers, mucous discharge from the mouth, nose and ears, cyanosis of comb and wattles, general depression, diarrhea and increased respiratory rate (Glisson et
al., 2008). Death losses from FC in chickens usually occur in laying flocks, because birds of this age group are more susceptible than younger chickens. Under natural conditions, mortality may range from only a few percent to nearly 100% (Glisson et al., 2008). In Bangladesh, the mortality rate reported was 25% to 35% in chickens and 11% in ducks (Choudhury et al., 1985; Baki et al., 1991). It is important to note that recovered birds may remain as carriers even after 9 weeks after infection (Kasten et al., 1997; Glisson et al., 2008).

Control of fowl cholera depends mainly on vaccination throughout the world including Bangladesh (Samad, 2000). Both live and inactivated (bacterins) vaccines have been attempted to control the disease (Glisson et al., 2008). Of them, inactivated vaccines are widely used as the organisms do not have any chance to be reverted to virulence to cause the disease (Hopkins and Olson, 1997). In Bangladesh, two vaccines are used very commonly that are produced locally and reported to provide good immunity (Akand et al., 2004; Rana et al., 2010). One is produced by the Livestock Research Institute, Mohakhali, Dhaka with a chicken isolate of \( \text{P. multocida} \) and another by the Bangladesh Agricultural University, Mymensingh with a duck isolate of \( \text{P. multocida} \) (PM-38) serotype 1 (X-73). Immune responses vary according to breed and rearing zone (Rana et al., 2010). Variation in the immunological response has been observed greatly in younger chickens (1-5 weeks of age) and birds vaccinated at 1 or 2 weeks of age appear to be consistent with the relatively low humoral antibody response (Dick and Avakian, 1991). Reports on the immune response and efficacy of locally prepared fowl cholera vaccines in chickens have been well documented in Bangladesh (Khan et al., 1994; Rahman et al., 2004a; Rahman et al., 2004b).

Presently, a research work is continuing at Bangladesh Agricultural University, Mymensingh, to develop a local broiler sire and dam lines through cross-breeding of Aseel and White Rock and Aseel (Choudhury et al., 1991). Field isolates of fowl cholera (PM-3) belonging to the serotype-1 (X-73) described by Chowdhury et al. (1986) was obtained from the stock culture of the Department of Microbiology of Hajee Mohammad Danesh Science and Technology University (HSTU), Basherhat, Dinajpur-5200, Bangladesh. Of these, the isolates of selected \( \text{P. multocida} \) (PM-38) organisms were cultured in blood agar media and kept in bacteriological incubator at 37°C for 24 hours and examined the purity of culture and subsequently subcultured in the same media for 24 hours. The isolated colonies were then inoculated in nutrient broth added with yeast extract 0.5gm per liter (0.5gm/L) and beef extract 2gm per liter (2gm/L) and incubated in 37°C for 48 hours for massive growth. Later on, formalin was added in the broth culture at the rate of 8 ml per liter (8ml/L) and after 24 hours alum was also added at the rate of 20gm per liter (20gm/L). Finally, it was dispensed in vials and stored at room temperature for future used. Thus, the vaccine is formalin inactivated alum-precipitated type. The safety and sterility of the vaccine was tested according to OIE manual.

### 2.2. Fowl Cholera Isolate

The field isolate of fowl cholera (PM-3) belonging to the serotype-1 (X-73) described by Chowdhury et al. (1986) was obtained from the stock culture of the Department of Microbiology of Hajee Mohammad Danesh Science and Technology University (HSTU), Basherhat, Dinajpur-5200, Bangladesh. Of these, the isolates of selected \( \text{P. multocida} \) (PM-38) organisms were cultured in blood agar media and kept in bacteriological incubator at 37°C for 24 hours and examined the purity of culture and subsequently subcultured in the same media for 24 hours. The isolated colonies were then inoculated in nutrient broth added with yeast extract 0.5gm per liter (0.5gm/L) and beef extract 2gm per liter (2gm/L) and incubated in 37°C for 48 hours for massive growth. Later on, formalin was added in the broth culture at the rate of 8 ml per liter (8ml/L) and after 24 hours alum was also added at the rate of 20gm per liter (20gm/L). Finally, it was dispensed in vials and stored at room temperature for future used. Thus, the vaccine is formalin inactivated alum-precipitated type. The safety and sterility of the vaccine was tested according to OIE manual.

### 2.4. Vaccination of Chicken

Fowl cholera vaccine was prepared in the laboratory at the dose rate of \( 5 \times 10^7 \text{CFU} \) (colony forming unit). For this, the isolates of selected \( \text{P. multocida} \) (PM-38) organisms were cultured in blood agar media and kept in bacteriological incubator at 37°C for 24 hours and examined the purity of culture and subsequently subcultured in the same media for 24 hours. The isolated colonies were then inoculated in nutrient broth added with yeast extract 0.5gm per liter (0.5gm/L) and beef extract 2gm per liter (2gm/L) and incubated in 37°C for 48 hours for massive growth. Later on, formalin was added in the broth culture at the rate of 8 ml per liter (8ml/L) and after 24 hours alum was also added at the rate of 20gm per liter (20gm/L). Finally, it was dispensed in vials and stored at room temperature for future used. Thus, the vaccine is formalin inactivated alum-precipitated type. The safety and sterility of the vaccine was tested according to OIE manual.

### 2.5. Passive Haemagglutination (PHA) Test

The test was used to determine antibody titers in chicken having vaccinated with fowl cholera vaccine as per method described by Chowdhury et al. (1986). The sensitivity and specificity of this PHA test procedure depends on the use of purified antigens. Antigens are coupled to chemically modified erythrocytes that readily react with specific antibodies and results haemagglutination. Sera of the immunized and control birds were collected and tested by PHA.

### 2.6. Challenge of Immunized Chickens

The challenge inoculum contains \( 5 \times 10^7 \text{CFU} \) as suggested by
Khan et al. (1994). For the challenge test, 5 (five) birds of each group of vaccinated and control were randomly selected.

2.7. Statistical Analysis

The mean and standard deviation (SD) of PHA titers were calculated by normal statistical model (Khan et al. 1994).

3. Results

Fowl cholera vaccine was administered at the dose rate of 1ml of 5×10^7 CFU as through intramuscular (IM) route via thigh muscle in each selected groups of chickens. Booster dose was given with the same dose and via the same route after 15, 30 and 45 days of primary vaccination in groups A, B and C, respectively.

The mean PHA titer is presented in the Table 1. The pre-vaccination PHA titers of sera samples of all vaccinated and control birds were nil. The primary vaccination induced slight rise of PHA titers which was ranging from 64 to 128 and booster vaccination at this stage triggered the production of PHA antibody titers very quickly ranging from 128 to 256. The mean PHA antibody titers on 15, 30, 60, 90, 120, 150 and 180 days post vaccination (DPV) in group A were 72.5±5.8, 230.4±13.6, 204.8±16.7, 115.2±6.8, 59.7±2.9, 32±0 and 11.2±1.0 in group B were 72.5±5.8, 53.3±4.0, 145.0±11.6, 73.5±5.8, 36.2±2.9, 19.2±1.7 and 4.8±0.4. The PHA titres gradually increased after 15 days of primary vaccination and then started to decline gradually and booster vaccination at this stage elucidated a rapid increase of PHA titres and continued to remain at a dependable immunity up to 5 month of post vaccination.

Result of challenge exposure demonstrated that alum-precipitated fowl cholera vaccine conferred 100% protection against challenge infection at 15 days post-booster when none of the unvaccinated control birds survived.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Vaccinations</th>
<th>Mean±SD passive haemagglutination titres at different days after vaccinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>A</td>
<td>Nil</td>
<td>72.5±5.8</td>
</tr>
<tr>
<td>B</td>
<td>Nil</td>
<td>72.5±5.8</td>
</tr>
<tr>
<td>C</td>
<td>Nil</td>
<td>72.5±5.8</td>
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<tr>
<td>D</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

4. Discussion

The pre-vaccination PHA titres of sera samples of all vaccinated and control birds were nil and were closely related with Mondal et al. (1988). The primary vaccination induced slight rise of PHA titres (ranging 64 to 128) and booster dose of vaccine at this stage triggered the production of PHA antibody titres (ranging 128 to 256). The findings were closely related with Coates et al. (1977) and Mondal et al. (1988). In group A, booster dose was given after 15 days of primary vaccination. The antibody titre was found to be highest 230.4±13.6 after 15 days of booster and sustained up to 60 days (204.8±16.7) and then PHA titre started to decline gradually but continued to maintain dependable immunity up to 5 month (32±0) of post vaccination. In group B booster dose was given after 30 days of primary vaccination. The PHA titres gradually increased after 15 days (73.5±5.8) of primary vaccination and started to decline at the end of 30 DPV (51.2±4.1). Boostering at this stage elucidated a rapid increase of PHA titres (170.6±16.1) and continued to remain at a dependable immunity up to 5 month (24.5±2.1) of post vaccination. In group C booster dose was given after 45 days of primary vaccination. The antibody titres gradually increased and started to decline very quickly at the end of 45 days (53.3±4.0). Booster dose at this stage triggered the production of antibody titres (145.0±11.6) and continued to remain at a dependable immunity up to 5 month (19.2±1.7) of post vaccination. The findings of this study in respect of impetuous production of PHA titres were similar with the observation of Collins (1977), Dua and Maheswaran (1978) and Mondal et al. (1988). These authors reported that inoculation of single dose of fowl cholera vaccine resulted in slight detectable rise of antibody titres and introduction of second dose of vaccine seven days later brought about an increase in such titre. In this respect, Chowdhury et al. (1985) observed that immune response of birds following single and dual vaccination indicated that dual vaccination at two weeks interval were more effective than single vaccination. A critical observation revealed that mean of PHA titres obtain with sera samples of group A and B were highest on all occasions compared with those of sera samples of group C. Based on the finding of the present investigation, it may be concluded that the vaccinal response were most satisfactory in birds of group A and B where booster dose was given after 15 and 30 days of primary vaccination. Bhasin and Biberstein (1968) and Mondal et al. (1988) found 100% protection against challenge infection of an alum-precipitated fowl cholera vaccinated birds at 5th week post vaccination.
References


