Efficacy of Montanide ISA-70-VG as Adjuvant to Fowl Cholera Vaccine

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DOI: 10.5455/jva.20150315015734
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Abstract

Water in oil emulsion fowl cholera vaccine was prepared using Montanide ISA-70-VG oil as alternative adjuvant to the used white mineral oil with span-80. The two vaccines were comparatively evaluated in SPF chickens. Each vaccine was inoculated in a group of 40 chickens while the third group of 40 chickens was kept as non-vaccinated control. It was noticed that the Montanide ISA-70-VG formulated vaccine induced mild transient local inflammation in comparison with those induced by the mineral oil formulated vaccine. Challenge test and application of indirect haemagglutination test on serum samples obtained from all chicken groups revealed that Montanide ISA-70-VG formulated vaccine induced earlier and higher immune response than that induced by the mineral oil formulated vaccine. So it could be recommended to use Montanide ISA-70-VG as adjuvant to fowl cholera vaccine aiming to provide early and high immune response able to protect chickens against fowl cholera infection.

Keywords: Pasteurella multocida, chicken, vaccination, isa70, whiterex oil.
Introduction

Fowl cholera (avian pasteurellosis) is a commonly occurring avian disease that can affect all types of birds and is often fatal and caused by infection with Pasteurella multocida. It typically occurs as a fulminating disease with massive bacteraemia and high morbidity and mortality (Derieux, 1978 and Rimler & Glisson, 1997). All avian species are susceptible to P. multocida, although turkeys may be the most severely affected. Often the first sign of disease is dead birds. Other signs include: fever, anorexia and depression, mucus discharge from the mouth, diarrhea, ruffled feathers, and drop in egg production coupled with smaller eggs, increased respiratory rate, and cyanosis at the time of death. Lesions that are often observed include: congested organs with serosal hemorrhages, enlarged liver and spleen, multiple small necrotic areas in the liver and/or spleen, pneumonia, and mild ascites and pericardial edema. Birds that survive the acute septicemic stage or those infected with organisms of low virulence may develop chronic fowl cholera, characterized by localized infections. These infections often involve joints, foot pads, tendon sheaths, sternal bursa, conjunctivae, wattles, pharynx, lungs, air sacs, middle ears, bone marrow, and meninges.

Lesions resulting from these infections are usually characterized by bacterial colonization with necrosis. Vaccination against infectious diseases is the corner stone in control of such diseases and contributed in eradication of them. Always there is a need to improve vaccine quality in order to provide safe and high potent vaccines. Mineral oil emulsion vaccines have been widely used to potentiate the antibody response and prolong duration of immunity (Cox and Coulter, 1997). Incomplete Freund’s adjuvant has been used extensively in many poultry vaccines (Zanella and Marchi, 1982) and used for over 30 years for production of fowl cholera vaccine in Egypt (Geneidy et al., 1971). Nowadays, Montanide ISA 70-VG was found to be incomplete seppic adjuvant type of mineral oil based on complex water in oil emulsion characterized by a low viscosity and easy to inject (Tehran et al., 2014).

This work was planned to compare the efficacy and immunity in between the prepared inactivated fowl cholera vaccine using Montanide ISA 70 VG oil as adjuvant and the used white oil.

Materials and Methods

Experimental Birds

One hundred and twenty (120) SPF eggs (were obtained from Kom Osheen farm in Fayoum) were incubated till hatching under complete hygienic measures and isolated in disinfected pins. All birds were confirmed to be seronegative for fowl cholera on the day of vaccination using indirect haemagglutination test.

Virulent Strain of Pasteurella Multocida

Local Pasteurella multocida serotype 5: A and D: 2 were kindly supplied and identified by Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia Cairo.

Binary Ethylene Amine (BEI)

BEI was used as an inactivator for preparation of inactivated fowl cholera vaccine according to Abou El-Saud et al., (1995).

Adjuvants

White oil

Whiterex oil FDA (Mobil Co.) with span (Honil Limited, London, BN. 9509A) and tween 80 (Sigma Co., St. Louis, Lot. 38H0550), they were mixed and used as adjuvant according to Stone et al., (1978).

Montanide ISA-70-VG

Montanide ISA 70 VG is a mineral oil based adjuvant which has been developed for the manufacture of water in-oil (W/O) emulsion provided by France Seppic Company, France.

Preparation of Inactivated Fowl Cholera Vaccine

Pasteurella multocida was cultivated in casamino acid medium (Bain, 1963) for 24 hours at 37°C with gentle aeration. After samples had been subjected to check purity and determine colony-forming unit (CFU) per ml, the culture was
inactivated for 24 hours at 37°C with BEI. Then the vaccine was standardized to contain \(10^6\) CFU/0.5 ml dose. The inactivated vaccine was divided into 2 portions where the white oil was added as adjuvant to the first portion according to Stone et al., (1978) while Montanide ISA 70 VG was added to the vaccine at the ratio 70(oil): 30(antigen) according to the manufacturer directions.

**Quality Control of the Prepared Vaccine**

The 2 prepared vaccine formulae were tested for sterility, safety and potency according to the standard international protocols as described by the British Veterinary Codex (1970) and Code of American Federal Regulation (1985).

**Vaccination**

All birds on 28 days old were seronegative for *P. multocida* after random samples examined using indirect HI test. These birds were divided into 3 groups as follows:

Group 1: of 40 birds was vaccinated with the prepared *P.multocida* vaccine with Montanide ISA 70 VG, as 0.5ml inoculated S/C for each bird.

Group 2: of 40 birds was vaccinated with the prepared *P.multocida* vaccine with the white oil as 0.5ml inoculated S/C for each bird.

*Each of group 1 and 2 received 2 doses with 4 weeks in between.*

Group 3: of 40 birds was kept without vaccination as a control.

**Indirect Haemagglutination Test (IHT)**

The test was carried out according to Carter and Rappy (1962) for evaluation of fowl cholera vaccine.

**Challenge Test**

The immunity of vaccinated and unvaccinated birds to *P. multocida* was tested by intramuscular challenge with 0.1ml of 24 hours old culture containing 100 LD50 of serotype 5: A and D: 2 as described by Heddleston and Rebres (1968). Clinical signs, mortality rates and gross lesions were recorded for 7 days post challenge. Reisolation of viable organisms was also tried from liver, heart blood and bone marrow of dead challenged birds. Each chicken group was subdivided into 2 subgroups (20 birds/subgroup) the first subgroup was challenged against serotype A: 5 while the second subgroup was challenged against serotype D: 2.

**Results and Discussion**

To achieve the goal of this work, to obtain an effective vaccine, the vaccine needs not only good antigen but also preferable adjuvant to enhance the antigen immunogenicity. Adjuvant used to enhance both of cellular and humeral immunity although it may induce side effects as inflammatory reactions; pain and tissue damage. Oil emulsion vaccine could promote antibody titer with prolonged immunity. However mineral oil long term stand at the site of injection causing inflammation and local tissue necrosis leading to lower commercial value of birds (Chun et al., 2011).

The present work includes preparation and evaluation of the white mineral oil and montanide ISA-70-VG as adjuvants to fowl cholera vaccine in a comparative manner aiming to determine the best one which achieve higher and best preferable antibody response in vaccinated chickens. The present obtained results showed that the Montanide ISA-70-VG formulated vaccine induced mild transient local inflammation in comparison with those induced by the white mineral oil formulated vaccine. It was concluded that Montanide ISA-70-VG caused obviously much less inflammatory reaction at the site of injection (Leenaars et al., 1998).

The demonstrated results in table 1 and 2 revealed that montanide ISA-70-VG induced earlier and higher immune response than that induced by the white oil as shown by passive haemagglutination test using serotype 5: A and D: 2; these findings were observed up to 12weeks post vaccination with the 1\(^{st}\) dose (8 weeks post vaccination with the 2\(^{nd}\) dose). Also table 3 and 4 revealed that Montanide ISA-70-VG provide higher protection rates against virulent serotype 5: A and D: 2 (95&90% respectively) than those obtained by the whit oil (90&85 respectively against the same two serotypes). In this respect it was found fowl cholera vaccine formulated with Mantonid ISA-70-VG provides good protection to immunized chickens as stated by Choi et al., (2008).
EFFICACY OF MONTANIDE ISA-70-VG AS ADJUVANT TO FOWL CHOLERA VACCINE

Table 1: Mean passive haemagglutination titers of fowl cholera antibodies in vaccinated chickens using serotype 5: A.

<table>
<thead>
<tr>
<th>Used vaccine</th>
<th>Mean passive haemagglutination titers (log&lt;sub&gt;2&lt;/sub&gt;/ml) of fowl cholera antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0WPV</td>
</tr>
<tr>
<td>ISA-70-VG</td>
<td>5</td>
</tr>
<tr>
<td>White oil</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*WPV= week post vaccination.

Table 2: Mean passive haemagglutination titers of fowl cholera antibodies in vaccinated chickens using serotype D: 2.

<table>
<thead>
<tr>
<th>Used vaccine</th>
<th>Mean passive haemagglutination titers (log&lt;sub&gt;2&lt;/sub&gt;/ml) of fowl cholera antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0WPV</td>
</tr>
<tr>
<td>ISA-70-VG</td>
<td>5</td>
</tr>
<tr>
<td>White oil</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*WPV= week post vaccination.

Table 3: Efficacy of used fowl cholera vaccines against serotype 5: A.

<table>
<thead>
<tr>
<th>Used vaccine</th>
<th>Number of died birds</th>
<th>Total number of survived</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24HPC</td>
<td>48HPC</td>
<td>72HPC</td>
</tr>
<tr>
<td>ISA-70-VG</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>White oil</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*HPC= hours post challenge.

Table 4: Efficacy of used fowl cholera vaccines against serotype D: 2.

<table>
<thead>
<tr>
<th>Used vaccine</th>
<th>Number of died birds</th>
<th>Total number of survived</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24HPC</td>
<td>48HPC</td>
<td>72HPC</td>
</tr>
<tr>
<td>ISA-70-VG</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>White oil</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

*HPC= hours post challenge.

So it could be concluded that Mantonid ISA-70-VG is a preferable adjuvant to fowl cholera vaccine and could be used as alternative to the whit oil providing better post vaccinal reaction and higher immune response with longe duration.

References