Evaluation the Quality of Oil-Emulsion Avian Influenza Subgroup H9N2 Vaccines in In-Vitro

Z. Rajabi,* H.Tayefi-Nasrabadi, A.B.Syofi-Khojin

Faculty of Veterinary Medicine, University of Tabriz, Tabriz-Iran

Abstract

The use of vaccines in poultry to control avian influenza viruses, especially mildly pathogenic avian influenza viruses has been increasing in recent years. In order to recommend any of the vaccines, it is important to evaluate the quality of them with an appropriate and rapid method. Three oil-emulsion vaccines commercially available against avian influenza viruses in Iran were evaluated for their quality by in-vitro assays. Total antigen was recovered from each vaccine by a method called aqueous partition. To normalize the immune response to the amount of the total protein available in each dose of each vaccine, the amount of extracted total protein and the amount of recovered hemagglutination activity were determined. Results showed that the amount of recovered total protein and activity of the recovered HA, were different in the vaccines tested. This difference was recorded for each dose of the vaccine used in the study. Also we showed that the hemagglutination inhibition activity of the antisera were different. It seems that the main reason for the low quality of the two vaccines compared to the other one is the low density of viral proteins in each dose of them.

Key words: Avian; Influenza vaccines; Oil-Emulsion vaccines; In-Vitro Evaluation

1. Introduction

Influenza is a highly contagious viral disease which infects birds, humans and other mammalians. The agent of disease belongs to the family Orthomyxoviridae which was isolated and identified in 1933 (6,9). Influenza virus type A has been isolated from wild and domesticated birds throughout the world (3). Three subtypes of Avian Influenza Viruses (AIVs), H5N1, H7N7, and H9N2 have directly infected humans (6,12). H9N2 subtype which is a mildly pathogenic virus spread worldwide in 1994-1999 (1); Evolution of H9N2 subtype (10), its genetic relation with H5N1 subtype (5), and its isolation from human, together (7,13), confirmed that the control of H9N2 viruses in poultry is important.

The use of oil-emulsion (OE) vaccines to prevention and control of avian influenza (AI) was rapidly increased after studies between1969-1978. These vaccines stimulate humoral immunity in the host and protect it against the challenge with acute viruses such as viscerotropic velogenic Newcastle disease and highly pathogenic avian influenza (HPAI) viruses (16). Vaccination of poultry with OE vaccines against AIVs especially mildly pathogenic viruses (MPVs) has been increasing in recent years.

The amount of viral proteins especially density and activity of hemagglutinin (HA) protein in AI OE-vaccines has the main role in immunogenicity and efficacy of the vaccines (4,16,17). With extraction of antigen mass of an AI OE-vaccine and determination of recovered HA activity and the amount of extracted total protein, it is possible to predict the quality and protective efficacy of the vaccine instead of using vaccination and challenge trials which is a time-consuming and expensive method, concerning ethical issues as well.

* Corresponding author's email: rajabi@tabrizu.ac.ir, Tel: +98411-3392342, Fax: +98411-3357834

© 2010 Published by Elsevier Ltd.

doi:10.1016/j.provac.2010.03.005
In this study antigen mass and the total protein of some AI OE-vaccines were extracted and the activity of recovered HA and the amount of total protein determined and compared with the serologic responses caused in chickens by each vaccine.

2. Vaccines

Three commercially available inactivated AI subtype H9N2 oil-emulsion vaccines (A, B, and C) used for this study.

3. Extraction of the antigen mass by aqueous partition method

To separate and measure aqueous phase of the OE-vaccines, 5 ml of each vaccine mixed with 3 ml of N-hexanol and centrifuged for 15 min at 1000 g, 4°C. For the extraction of the antigen mass from the intact vaccines, 12 doses of each vaccine (2.4 ml of vaccine A, 3.6 ml of vaccine B, and 3 ml of vaccine C) were added to centrifuge tubes containing phosphate-buffered saline (PBS), 1.33 ml, 2.63 ml, and 3 ml respectively. Then, placed in an ice bath, and cooled to 0°C (the ratio between PBS volume and aqueous phase volume was equal in the three vaccines). The contents were mixed using a Homogenizer (IKA ULTRA-TURRAX® T 18 basic) at 20000 rpm for 50 seconds on ice. The mixtures were then centrifuged at 1000 g for 15 min at 4°C to separate PBS fraction from the oil fraction of the OE-vaccine. HA activity of PBS fraction for each vaccine was determined and expressed as HA titer. Extraction of antigen mass was conducted 2 days before vaccination.

4. Quantification of extracted protein

Protein concentration of PBS fraction of the vaccines was determined by the method of Lowry et al (1951) using crystalline bovine serum (11). First 25 ml of 2% sodium carbonate was mixed with 25 ml of 0.1% sodium hydroxide solution to prepare Solution A. Then, 5 ml of Copper sulfate solution was mixed with 5 ml of 1% sodium potassium tartarate solution to prepare Solution B. 50 ml of solution A was mixed with 1 ml of solution B to prepare solution C. To quantify total protein, 0.2 ml extracted PBS from each vaccine was mixed with 1 ml Solution C and was incubated for 10 min at room temperature. 0.1 ml of Folin - Ciocalteau reagent solution (1N) was added to the test tubes and was vortexed briefly. After 30 min incubation, the absorbance of it determined at the wave length 550 nm. Standard curve prepared in the concentrations of 25, 50, 100, 200, and 400 μg/ml.

5. Vaccine efficacy study

One hundred day-old broiler chickens were obtained from a commercial hatchery and reared by standard practices. Before vaccination they were randomly divided equally into four groups (I, II, III, and IV) and placed in separate cages (25 chickens per each group) in an isolation room. The group I, II, and III received vaccine A (0.2 ml/dose), vaccine B (0.3 ml/dose), and Vaccine C (0.25 ml/dose) respectively in the dorsal cervical region via the subcutaneous route at 11 days of age. The group IV received PBS (0.3 ml) as control. Blood samples were taken at five weeks post-vaccination for haemagglutination inhibition (HI) test. HI titers expressed as HI mean titers based on 25 chickens per group.

6. HI assay method

Sera were tested for HI antibodies to avian influenza H9N2 using 4 HA units of the antigen as described by Beard & Wilkes (1973) (2).

7. Statistical analysis

Data were analyzed by spss package (16.2.2) using (Npar tests) Kruskal-Wallis test.

8. Results and Discussion

The results from this study showed that the aqueous partition method retrieves antigen mass from the inactivated AI subgroup H9N2 OE-vaccines, and also indicated that in three examined vaccines there is a good correlation among recovered hemagglutination activity, extracted total protein, and antibody response in each vaccine (Table 1).
Table 1. Correlation among recovered haemagglutination titters, extracted total protein, and HI titters.

<table>
<thead>
<tr>
<th>Vaccines&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HA titters&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Total protein&lt;sup&gt;d&lt;/sup&gt; (µg/ml)</th>
<th>HI m&lt;sup&gt;e&lt;/sup&gt; at 5-wk post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>4.8</td>
<td>5.08</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>2</td>
<td>2.76</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>7</td>
<td>6.24</td>
</tr>
<tr>
<td>D&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>0.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Inactivated avian Influenza H9N2 oil-emulsion vaccines.  
<sup>b</sup>PBS as a negative control of vaccines.  
<sup>c</sup>Recovered haemagglutination titer.  
<sup>d</sup>Extracted vaccine total protein.  
<sup>e</sup>Haemagglutination-inhibition mean titters based on a total of 25 chickens/group.

Incomplete control of MPAI viruses and circulation of viruses in poultry houses may predispose antigen changes and possibly increase pathogenicity (8). Most commercial AI OE-vaccines only reduce or inhibit clinical signs and or mortality and do not prevent viral replication and shedding of AIVs completely (7).

Numerous studies have indicated that the efficacy and immunogenicity of AI OE-vaccines depend on the amount of antigen mass especially HA protein (16,17). Garcia and others have shown that to prevent AI clinical signs a dose of vaccine must have approximately 0.4 µg hemagglutinin (4). Other study have shown that in order to induce immunity and prohibit viral shedding, the amount of hemagglutinin must be 0.5 µg (16).

Stone has shown that by using aqueous partition method, it is possible to recover HA protein and determine HA activity from OE Newcastle disease vaccines (14).

Both hemagglutinin and neuraminidase (NA), especially hemagglutinin, induce protective antibodies; in addition, there are some reports that indicate internal proteins mainly nucleoprotein (NP) of AI viruses induce antibodies which decrease titers of influenza replication in lungs (15), there is also a direct relationship between quantity of the whole virus and the hemagglutinin in a vaccine. It is also clear that in the PBS fraction of the vaccines, there are some recovered internal proteins including, NA, and matrix (M), besides hemagglutinin antigen. Therefore, it is possible to evaluate the quality of a vaccine by determining the extracted total protein instead of using vaccination and challenge trials. Therefore, by extracting and determining of total protein in a vaccine, one could acquire information about the potency of the vaccine. The results from this study showed that the aqueous partition method retrieves antigen mass from the AI subgroup H9N2 OE-vaccines, and also indicated that immune responses to the three examined vaccines are significantly different (P=0.000) at the same doses and the amount of extracted total protein and activity of the recovered HA are higher in vaccine C than the two others (vaccine A and B), (table 1). Based on the results of viral protein extraction and the activity of recovered HA, it seems that the main reason of low efficacy for the two vaccines is the low density of the viral proteins in vaccines.

9. Acknowledgments

The authors thank Dr. Biorani for statistical analysis of the data. This research was supported by Research Council of University of Tabriz.

10. References


