Journal of Animal and Veterinary Advances 7 (5): 604-606, 2008
ISSN: 1680-5593
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Determination of Hemagglutination Activity and Total Protein Recovered from Oil-Emulsion Avian Influenza Vaccines as a Prediction of Efficacy

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Abstract: The use of vaccination in poultry to control AI viruses, especially Mildly Pathogenic Avian Influenza (MPAI) viruses has been increasing in recent years. The amount of antigen mass and hemagglutination activity of Oil-Emulsion (OE) vaccines is important for the control of Avian Influenza (AI) viruses. With extraction of antigen mass of an AI OE-vaccine and determination of Hemagglutination (HA) titers and total protein; it is possible to predict the quality and protective efficacy of the vaccine. In this study, antigen mass was recovered from three commercially available AI OE-vaccines by aqueous partition method, then, the amount of extracted total protein and recovered HA activity were compared with the serologic responses caused in chickens by each vaccine. The results 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 HA activity, extracted total protein and antibody response in each vaccine. This study also showed that Determination of both recovered HA activity and extracted total protein let us know more about the efficacy of an OE-vaccine.

Key words: Avian influenza, oil emulsion vaccines, hemagglutination activity, MPAI, avian, species

INTRODUCTION

The immunogenicity and efficacy of avian influenza Oil-Emulsion (OE) vaccines are well documented in a variety of avian species (Swayne *et al*., 2001; Karunakaran *et al*., 1987; Stone, 1987), therefore, vaccination can be a good tool for the control of Avian Influenza (AI) viruses if coupled with limitation and expanded biosecurity (Capua and Marangon, 2003). The use of vaccination in poultry to control AI viruses, especially Mildly Pathogenic Avian Influenza (MPAI) viruses has been increasing in recent years.

The efficacy of an inactivated AI whole virus vaccine directly depends on antigen mass especially hemagglutinin and its Hemagglutination (HA) activity (Swayne *et al*., 1999; Capua and Marangon, 2003). With extraction of antigen mass of an AI OE-vaccine and determination of recovered HA titers and 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. In this study, antigen mass was recovered from AI OE-vaccines and the amount of extracted total protein and recovered Hemaglutination (HA) activity were compared with the serologic responses caused in chickens by each vaccine.

MATERIALS AND METHODS

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

Extraction of antigen mass by aqueous partition method: To separate and measure aqueous phase volume of the OE-vaccines, 5 mL of each vaccine mixed with 3 mL of N-hexanol and centrifuged (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 appropriate amounts of Phosphate-Buffered Saline (PBS) (1.33, 2.63 and 3 mL,

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respectively), then, placed in an ice bath and cooled to 0°C (ratio of PBS volume to aqueous phase volume was equal in the three vaccines). The contents were mixed by a Homogenizer (IKA ULTRA-TURRAX® T 18 basic) at 20000 rpm for 50 sec. The mixtures were then centrifuged at 1000 g for 15 min at 4°C to allow separation of PBS fraction from the OE-vaccine. HA activity of PBS fraction of each vaccine was determined and expressed as the reciprocal of the HA titer. Extraction of antigen mass was done 2 days before vaccination.

Determination of extracted total protein: Protein concentration of PBS fraction of the vaccines was determined by the method of Lowry *et al.* (1951) using crystalline bovine serum as standard.

Vaccine efficacy study: One hundred day-old broiler chickens were obtained from a commercial hatchery and reared by standard practices. Before vaccination they randomly divided 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, group II and group III received vaccine A (0.2 mL dose^{-1}), vaccine B (0.3 mL dose^{-1}) and Vaccine C (0.25 mL dose^{-1}) respectively in the dorsal cervical region via the subcutaneous route at 11 days of age, the group IV received PBS (0.3 mL dose^{-1}) as a control group. Blood samples were taken at five weeks post-vaccination for hemagglutination inhibition (HI) serology. HI titers expressed as reciprocal of HI geometric mean titers.

HI assay method: Sera were tested for HI antibodies to avian influenza H9N2 using 4 HA units of antigen.

RESULTS

Aqueous phase of the vaccines: The aqueous phase of the vaccine A, B and C was 1.2 mL/5 mL, 1.6 mL/5 mL and 2.2 mL/5 mL, respectively.

Recovered hemagglutination titers: Table 1 shows the recovered HA titers, extracted total protein and antibody response to the commercial vaccines tested. The HA titers which recovered from vaccines by the aqueous partition technique ranged from 1-8. Low HA titer was correlated with low total protein and low potency (vaccine B), as indicated by HI titers. High HA titer was correlated with high total protein and high potency (vaccine C).

Extracted total protein: Table 1 also shows the recovered HA titers, extracted total protein and antibody response to the commercial vaccines tested. The extracted total protein ranged from 2-7 μg mL^{-1}. Low total protein was correlated with low recovered HA titer and low potency (vaccine B), as indicated by HI titers. High total protein was correlated with high recovered HA titer and high potency (vaccine C).

Table 1: Correlation among recovered hemagglutination titers, extracted total protein and HI titers

Vaccines[a]	HA titers[c]	Total protein[d] (μg mL^{-1})	HI GMT[e] at 5 week post-vaccination
A	4	4.8	34.30
B	1	2	6.73
C	8	7	76.10
D[b]	-	-	<3

[a]Inactivated avian Influenza H9N2 oil-emulsion vaccines, [b]PBS as a negative control of vaccines, [c]Recovered hemagglutination titer, [d]Extracted vaccine total protein, [e]Reciprocal of hemagglutination-inhibition geometric mean titer based on a total of 25 chickens/group

HI Geometric Mean Titer (GMT): The HI titers of vaccinated chickens ranged from 6.73-76.10, which indicate the potency and efficacy of the vaccines. Antibody response to vaccine C is greater than vaccine A and vaccine B (Table 1).

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). Antibody to hemagglutinin antigen of AI viruses mainly protect chickens from death and clinical signs (Swayne and Halvorson, 2003), therefore, the quantity and quality of hemagglutinin in an inactivated vaccine are two most reliable criteria to predict the vaccine efficacy. Aqueous antigens of OE-vaccines usually have been combined with the oil and emulsifiers. It is possible to use aqueous partition method or freeze-thaw method to recover antigen mass from OE-vaccines (Stone, 1985). This study showed that the aqueous partition method, similar to the previous study on inactivated Newcastle Disease (ND) virus vaccines can retrieve hemagglutinin antigen from inactivated AI virus vaccines. This study also showed that when 1 part aqueous phase is partitioned with about 2.29 parts of PBS the measurable HA activity is recovered from AI OE-vaccines by aqueous method, while in the previous study when 1 part aqueous phase is partitioned with 9 parts of PBS the measurable HA activity is recovered from ND OE-vaccines. Two major factors may be caused this difference: either the antigen mass of AI OE-vaccines was at the least or the hemagglutinin antigen has been destroyed in the process of OE-vaccines production. Therefore, the aqueous partition method could be useful, by saving time and cost,

to estimate OE-vaccine efficacy before application, but it needs more work on all OE-vaccines of known formulations to show the optimum conditions, the limits and sensitivity of the partition method, as recommended by Stone (1985).

In spite of the fact that 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 through the late steps of the infectious process (Swayne and Halvorson, 2003), as well as there is a direct relationship between quantity of the whole virus and hemagglutinin in a vaccine. It is also clear that in the PBS fraction of the vaccine, there are extracted internal proteins, NA and Matrix (M), besides recovered hemagglutinin antigen. Therefore, it is possible to evaluate the quality of a vaccine by determination of extracted total protein. The results from this study showed that in the PBS fraction there is extracted total protein, as well as, it has a good correlation with recovered HA titers and antibody response to each vaccine (Table 1). AI vaccines for poultry are not highly purified. The AI viruses which used in AI vaccines usually propagated in embryonated chicken eggs, therefore the total protein of the vaccines includes antigen mass and some egg proteins, however, this study indicated that egg proteins do not affect on the positive correlation between total protein and antibody response, because the aqueous phase of the vaccines was diluted and killed AI viruses which used in these vaccines are propagated in embryonated chicken eggs.

Although, determination of recovered HA titers may help to estimate OE-vaccine efficacy, but this method can not describe the reason of high or low efficacy of the vaccine. Determination of both recovered HA activity and extracted total protein let us know more about the efficacy of an OE-vaccine.

ACKNOWLEDGEMENTS

The authors thank Saeid Mostofi for his gift of H9N2 antigen. This research was supported by Research Council of Tabriz University.

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