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Original Article

Evaluation of Quality of Two Commercial Oil-Emulsion Newcastle Disease Vaccines In-vivo and In-vitro

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Abstract

Background and Aims: Newcastle disease (ND) is a highly contagious disease in poultry with economic losses in the world. Vaccination is one of the most important ways for prevention and control of NDV, but there are reports of ND outbreaks in vaccinated chickens. Poor quality of the vaccines is one of the reasons of vaccine failure. In this study the quality of two commercial oil-emulsion Newcastle disease virus (OE-NDV) vaccines was evaluated in in-vitro and in-vivo.

Materials and Methods: For in-vitro study, the amount of total protein and quantity of retrieved hemagglutination activity (HA) of the vaccines were recovered and measured, then, the amount of recovered total protein and retrieved HA were compared with the serologic responses induced in chickens by each vaccine(in-vivo study).

Results: The results showed that the extracted total protein , recovered HA titer, and Mean HI titers to vaccine A are higher than vaccine B.The results also indicated that there is a good correlation between retrieved HA activity, extracted total protein and antibody response to vaccine A, but about vaccine B, there is no good correlation between total protein and retrieved HA activity and antibody response.

Conclusions: Quality of Vaccine B is lower than vaccine A, likely because of many reasons particularly improper storage of the vaccine, inappropriate harvesting of egg allantoic fluid and inactivation of NDV.

Keywords: Hemagglutination activity, Total protein, vaccine potency, Hemagglutination inhibition.

Introduction

Newcastle disease virus (NDV) which is a member of the paramyxoviridae family causes highly contagious disease in poultry and other birds. It widespreads in all over the world causing economic problems (1).

Although, vaccination is one of the most important ways for prevention and control of NDV (1-7), there are reports of ND outbreaks in vaccinated chickens (8-10). The poor quality of the vaccines is one of the reasons of vaccine failure.

Evaluation of potency of inactivated NDV vaccines usually is achived in vivo by vaccination and subsequent HI test and challenge of birds (11).

In addition of in vivo study, determination of retrieved HA activity (12,13) and determination of the extracted total protein of inactivated NDV vaccines, will help us get more information for developing of the vaccines. The previous studies indicated that it is possible to extract total protein from inactivated avian influenza vaccines and retrieve HA activity with aqueous partition method (14,15). In this study we evaluated the the quality of two commercial inactivated OE-NDV vaccines in in-vivo, and in-vitro by both

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the determination of the amount of extracted total protein and determination of the quantity of regained hemagglutination (HA) activity.

Methods

Vaccines. Two available commercial inactivated NDV vaccines, A and B, were applied for this study.

Aqueous partition method. For determining the aqueous phase of the OE vaccines, 5 mL of each vaccine mixed with 3 mL of 1-hexanol in a centrifuge tube at room temperature and centrifuged at 1000 g for 15 min at 4˚C to separate and measure aqueous phase volume. To recover the HA antigen, 45 doses of each vaccine, 4.5 mL of the vaccine A and 22.5 mL of the vaccine B, were separately added to a centrifuge tube that contained the appropriate quantities of PBS, 4.5 mL and 22.5 mL respectively, Aqueous phase of each vaccine, and PBS to aqueous phase ratios determined the volume of PBS. They were placed in an ice bath, and cooled to 0˚C. The contents were mixed by a Homogenizer (IKA ULTRA-TURRAX® T 18 basic) at 20000 rpm for 50 seconds at 4˚C. The mixtures were then centrifuged at 1000 g for 15 min at 4˚C to allow separation of PBS fraction from the OE vaccine (13). Recovery of HA activity of the vaccines was done 2 days before vaccination.

Hemagglutination Assay. Hemagglutination test was achived as recommended by (16). PBS fraction of the vaccines was used for determining hemagglutination activity of retrieved hemagglutinin.. HA activity determined and expressed as reciprocal dilution titer.

Determination of the extracted total protein. Protein concentration of PBS fraction of the vaccines was determined by the method of Lowry et al.(17) using crystalline bovine serum albomin as standard. Briefly, 2% Na2CO3 mixed with 0.1 normal NaOH and called A reagent, 0.5% CuSO4.5H2O mixed with 1% sodium tartrate and called B reagent, 50 mL of reagent A mixed with 1 mL of reagent B and called C reagent. For measuring extracted total protein of the vaccines, 0.2 mL of PBS fraction of each vaccine separately mixed with 1 mL of reagent C, after 10 min incubation at room temperature, 0.1 mL of 1 normal of Folin-Ciocalteu reagent was added and completely mixed. After 30 min the sample was read in a spectrophotometer which adjusted to a wavelength of 550 nm. Standard curve prepared in concentrations of 25, 50, 100, 200 and 400 µg/mL of BSA.

Efficacy of the vaccines in broiler chickens. Seventy two day-old broiler chickens (Ross 308®) were obtained from a commercial hatchery and reared by standard practices. Before vaccination they randomly divided into three groups (I, II and III), each group had two subgroups, and placed in separate cages (12 chickens/cage). Group I and group II received vaccine A (0.1 mL/ dose), and vaccine B (0.5 mL/dose) respectively in the dorsal cervical region via the subcutaneous route at 11 days of age, the group III received PBS (0.5/dose) as a control group. Blood samples were taken at five weeks old, approximately three weeks post-vaccination, for HI serology.

Hemagglutination inhibition assay. This test was achived as recommended by Alexander et al. (18). Mean NDV HI titers of each group was determined and expressed as reciprocal.

Statistical analysis. One-way ANOVA and Duncan multiple range tests were used for analysis of HI titers. The SPSS statistics, 22 version, was used for statistical analysis.

Results

Aqueous phase of the vaccines. OE-vaccines consist of two phases, oil phase and aqueous phase, antigens of OE- vaccines are in the aqueous phase. In this study the aqueous phase of both vaccines was 1.2 mL/5mL of the each vaccine. These results indicated that aqueous to oil ratios are different between each dose of the vaccines.

Recovered haemagglutinin titers. The HA titers of vaccine A and B which recovered by the aqueous partition technique were 9 and 2 respectively (Table 1). These results showed that the aqueous partition method, similar to a previous study on inactivated NDVs vaccines (Stone 1985) can retrieve HA antigen from commercial inactivated NDV vaccines; in

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Table 1: Comparison of potensy of the vaccine A and B in in-vitro and in-vivo.

Vaccinesa	HA titerc	Total proteind µg/mL	HI e at 24-day post vaccination
A	9	2.65	4.5 ± 0.31c
B	2	2.50	3.4 ± 0.29b
Cb	0	0	0.1 ± 0.07a

addition, the amount of active hemagglutinin of vaccine A is much more than vaccine B per dose.

Extracted total protein. The extracted total protein of vaccine A and B was 2.65 and 2.50 µg/mL of PBS fraction of the vaccines respectively. These results indicated it was possible to determine the extracted total protein , and the extracted total protein of vaccine A is more than vaccine B.

HI mean titers. The mean HI titers of chickens which vaccinated with vaccine A and B were 4.6 and 3.4 respectively, which statistically there is significant difference between them ($P<0.05$). These results indicated that the efficacy of vaccine A was more than vaccine B (Table 1).

Discussion

NDV occurs in poultry industry of developed and developing countries despite the widespread use of vaccination (19-21), therefore, it is necessary to continue evaluation and developing of NDV vaccines. Immunogenicity of NDV inactivated vaccines depends on different factors specially inactivating agents, type of adjuvant and content of antigens (22,23). At least 90% variations in HI antibody titers after NDV vaccination could be related to differences in NDV-HN and F content of the vaccines (24).

The influence of intact hemagglutinin protein of NDV vaccines in immunity against Newcastle disease has been confirmed by many researchers (1,11,12). A NDV inactivated vaccine must have 400-600 intact HA units per dose for prevention of Newcastle disease (11), which has direct correlation with hemagglutinin titers. In this study, the results of recovered haemagglutinin titers indicated that the content of NDV and indirectly content of intact HA in vaccine A is more than vaccine B, because the recovered HA titer of vaccine A is 4.5 times higher than vaccine B.

Commercial NDV inactivated vaccines usually produced by growing virus in eggs, and then treating whole virus with inactivating agents (24), therefore the extracted total protein, in addition of hemagglutinin protein may also contain other immunogenic proteins, and non-immunogenic proteins of NDV, but we know that there is high correlation between content of total protein and content of immunogenic proteins of the virus. In this study, there was not much difference between the total protein of vaccine A and B, in spite of much difference in retrieved HA activity and antibody response. It means probably either the harvesting of allantoic fluid from eggs or inactivation of NDV or conditions of storage had been incorrect in vaccine B; for any reason, quantity of inactivated whole NDV and subsequently recovered HA activity of the vaccines were different, in spite of using the equal doses and the same ratios of PBS to aqueous part of vaccines for extraction.

There is a good correlation between presence of HI antibody titers and protection from ND at day of challenge (2,12), therefore postvaccinal serology, usually HI test, is used to evaluate an adequate immune response by the bird to vaccination (1). The results of this study indicate that the immunogenicity of vaccine A is more than vaccine B.

The presence of adequate amount of intact hemagglutinin and other immugenic antigens of NDV, indirectly total protein of NDVs, in inactivated NDV vaccines are necessary for good protection (2,11,24). There are reports that indicate there is high correlation between the log NDV –HN content of OE-NDV

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vaccines and HI antibody titers (12), and between antibody response and HAU per vaccine dose (11). The previous studies also indicated that there is high correlation between extracted total protein of inactivated avian influenza vaccines and retrieved HA activity and HI titers (15).The present study indicate that there is a good correlation between retrieved HA activity, extracted total protein and antibody response to vaccine A, but about vaccine B, there is no good correlation between total protein and retrieved HA activity and antibody response.

Conclusion

Quality of Vaccine B is lower than vaccine A, likely because of many reasons particularly improper storage of the vaccine, inappropriate harvesting of egg allantoic fluid and inactivation of NDV.

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