

Original Article

Evaluation and Comparison of the Potential Immunogenicity of Two Commercial Inactivated Bivalent Newcastle and Avian Influenza Vaccines in SPF Chicken

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ABSTRACT

In the present study, the potency and immunogenicity of the inactivated bivalent vaccine of Newcastle disease (ND) and avian influenza (AI) produced by Razi institute in Iran were compared with a similar imported vaccine administered by standard methods to specific-pathogen-free (SPF) chicken. A total of 150 twenty-one-day-old SPF chickens were used for evaluating Razi and imported inactivated H9N2/ND vaccines. The chickens were divided into eight groups. The subjects in groups 1 and 3 were vaccinated with 0.3 ml/bird by subcutaneous route in the back of the neck with Razi and imported vaccines, respectively. Chickens in group 2 received Razi vaccine based on the recommended dose of the manufacturer (0.2 ml/bird) by the same route. The birds in groups 4 and 5 received 0.01 ml/bird of Razi and imported vaccine, respectively. Groups 7 and 8 were considered for studying the safety of the two vaccines and received a double amount of the full dose of vaccines. Moreover, group 6 was regarded as the negative control. Sera were collected weekly from each chicken for antibody analysis against Newcastle disease virus (NDV) and AI virus (AIV) and the study continued for 15 weeks after vaccination. An immunological evaluation was carried out using haemagglutination inhibition (HI) antibody test against NDV and AIV. The results showed that up to 15 weeks after vaccination, the Razi vaccine induced a higher level of protective antibody against AI and ND in comparison with the imported ones at the dose of 0.3 ml/bird. The mean HI titer was significantly different between Razi vaccine and imported vaccine at the dose of 0.3 ml/bird. There was no statically significant difference between Razi vaccine (0.2 ml/bird) and the imported vaccine (0.3 ml/bird) against NDV and AIV. According to the findings, 15 weeks after vaccination, HI titers were still detectable at a high level. The mean HI titer was found as 5.2 log₂ against NDV and 5 log₂ against H9N2 with Razi vaccine (0.3 ml/bird). In addition, the mean HI titer with Razi vaccine (0.2 ml/bird) was 4.1 log₂ and 4.7 log₂ against ND and AI, respectively. In summary, our results indicated that Razi inactivated vaccine (0.3 ml/bird) induced a strong and rapid antibody response in vaccinated chickens and is more effective in chicken against AIVs and NDVs, in comparison with the imported vaccine.

Keywords: Avian influenza, H9N2 subtype, Inactivated vaccine, Newcastle disease

L'évaluation et la Comparaison de l'immunogénicité Potentielle de Deux Vaccins Commerciaux Bivalents Inactivés de Newcastle et de L'influenza Aviaire chez des Poulets SPF

Résumé: Dans cette étude, l'activité et l'immunogénicité du vaccin bivalent inactivé de la maladie de Newcastle (ND) et de l'influenza aviaire (IA) produit par l'institut Razi en Iran ont été comparées à un vaccin importé similaire. Dans ce but les vaccins ont été administrés par le biais de méthodes standard à des poules exempts d'agents pathogènes spécifiques (SPF). Au total, 150 poulets SPF âgés de 21 jours ont été utilisés pour évaluer les vaccins H9N2 / ND inactivés de l'institut Razi ainsi que ceux importés. Les poulets ont été divisés en huit groupes. Les sujets des groupes 1 et 3 ont été vaccinés par voie sous-cutanée à l'arrière du cou avec 0,3 ml / oiseau des vaccins Razi et importés, respectivement. Les poulets du groupe 2 ont reçu le vaccin Razi selon la

dose recommandée par le fabricant (0,2 ml / oiseau) par la même voie. Les oiseaux des groupes 4 et 5 ont reçu 0,01 ml / oiseau de vaccin Razi et importé, respectivement. Les groupes 7 et 8 ont été pris en compte pour étudier l'innocuité des deux vaccins et ont reçu une double quantité de la dose complète des vaccins. De plus, le groupe 6 était considéré comme le contrôle négatif. Des sérums ont été prélevés chaque semaine sur chaque poulet pour une analyse d'anticorps dirigés contre le virus de la maladie de Newcastle (NDV) et le virus de l'IA (AIV). L'étude a été poursuivie 15 semaines après la vaccination. Une évaluation immunologique a été réalisée en utilisant un test d'inhibition de l'hémagglutination (IH) contre le NDV et le VIA. Les résultats ont montré que jusqu'à 15 semaines après la vaccination, le vaccin Razi administré à une dose de 0,3 ml/oiseau, induisait un taux plus élevé d'anticorps protecteurs contre l'IA et la MN comparé au vaccin importé. Le titre moyen de l'IH était significativement différent entre le vaccin Razi et le vaccin importé. De plus, il n'y avait pas de différence statistiquement significative entre l'efficacité du vaccin Razi administré à 0,2 ml/oiseau et le vaccin importé injecté à 0,3 ml / oiseau contre le NDV et le VVA. Selon les résultats, 15 semaines après la vaccination, les titres d'IH étaient encore détectables à un niveau élevé. Le titre moyen IH était de 5,2 log₂ contre le NDV et de 5 log₂ contre le H9N2 pour le vaccin Razi (0,3 ml / oiseau). En outre, le titre moyen de l'IH associé au vaccin Razi (0,2 ml / oiseau) contre la MN et l'IA était respectivement de 4,1 log₂ et de 4,7 log₂. En résumé, nos résultats ont indiqué que le vaccin inactivé produit par l'Institut Razi et administré à la dose de 0,3 ml/oiseau induisait une réponse anticorps forte et rapide chez les poulets vaccinés et s'est avéré plus efficace dans la protection des poulets contre les AIV et les NDV comparé au vaccin importé.

Mots-clés: Influenza aviaire, Sous-type H9N2, Vaccin inactivé, Maladie de Newcastle

INTRODUCTION

The poultry industry, both industrial and traditional, is encountered with serious problems due to the development of various diseases, including Newcastle disease (ND) and avian influenza (AI). These two diseases are considered as the two most dangerous and most harmful diseases in poultry worldwide (Wang et al., 2016). Avian influenza virus (AIV) and Newcastle disease virus (NDV) are known as two pathogens of birds that cause economic loss for the poultry industry every year (Horimoto and Kawaoka, 2001; Alexander, 2003; de Leeuw et al., 2005; Banet-Noach et al., 2007; Wang et al., 2016). In spite of preventive measures and vaccination, the sporadic incidence of these diseases has been reported in industrial flock poultry (de Leeuw et al., 2005). Low pathogenic avian influenza virus (LPAIV) has been circulating in industrial poultry for two decades in Iran (Nili and Asasi, 2003). LPAIV has often caused slight to moderate mortality with apparent clinical signs that are characterized by depression, decrease in egg production, and respiratory signs (Lee et al., 2000; Capua and Alexander, 2004; Li et al.,

2005). Vaccination is considered to be the most effective way for prevention from infectious diseases in poultry with scientific and practical principles reducing the waste of time and money (Choi et al., 2008; Lee and Song, 2013). Vaccination against ND will ideally cause immunity to the infection and virus replication leading to the low levels of replication and repulsion of the virus. Nonetheless, this method can only protect the birds from the severe negative impacts of the disease. It should be noted that management and good health conditions can never be replaced with vaccination (Westbury, 1984). Vaccination against NDV and AIV is an important route for the prevention and control of AIV and NDV in poultry farms (Choi et al., 2008). The initial studies on inactivated vaccines showed proved them to be protective in birds (Beard and Easterday, 1967). Although inoculation of inactivated NDV and H9N2 LPAI vaccines is protective in broiler and layer breeder farms, diverse factors affect the response to the vaccine. The type of inactivated vaccines, species of avian in farms, and the dose of vaccine are important in terms of antibody response (Lee and Song, 2013; Zhao et al., 2017). With this background in mind, the purpose

of this study was to evaluate the potency and immunogenicity of inactivated dual vaccine of ND and AI produced by Razi institute in Iran at the doses of 0.3, 0.2, and 0.01 ml, in comparison with the similar imported vaccines. This study examined the effect of Razi and imported inactivated bivalent ND and AI vaccines with different doses on the immunization of poultry.

MATERIAL AND METHODS

Birds. This study was conducted on 150 one-day-old specific-pathogen-free (SPF) laying chickens. On the 21st day, the chickens were randomly assigned into eight groups, including seven experimental and one control groups with 10 chickens in each. Each group was reared in laboratory animal room of Razi Vaccine and Serum Research Institute. Food and water were available *ad libitum*.

Experimental Design. At the age of 21 days, the chickens in group 1 were vaccinated with Razi inactivated ND and AI (0.3 ml/bird) vaccine by subcutaneous route in the back of the neck. The chickens in group 2 were vaccinated with 0.2 ml/bird by the same route. The birds in group 3 received the imported inactivated ND/AI vaccine at the manufacturing company recommended dose (0.3 ml/bird) by the same route. The subjects in groups 4 and 5 were vaccinated with Razi and imported vaccines at the dose of 0.01 ml/bird, respectively (Table 1). Moreover, groups 7 and 8 were considered for studying the safety of the vaccines and received double full vaccine dose. Blood samples were taken from the chickens before vaccine injection to ensure that there was no maternal viral antibody titer. After injection, blood samples were collected from all groups for blood serum separation followed by HI test for all groups (except for groups 7 and 8) up to 15 weeks of age. Furthermore, in order to evaluate the potency of a vaccine, the blood samples of all chickens from groups 1 to 6 were collected weekly for 15 weeks. The serum samples were taken for HI testing. Groups 7 and 8 were

considered for studying the possible tissue reactions after 3 weeks from the injection time.

Table 1. Experimental design for the evaluation of the immunogenic potency of the two commercial NDV/AI inactivated oil vaccines in SPF chickens

Group No	Chicken	Age/day	Type of Vaccine	Dose/ml
1	20	21	Razi	0.3
2	20	21	Razi	0.2
3	20	21	Imported	0.3
4	20	21	Razi	0.01
5	20	21	Imported	0.01
6	10	21	PBS	0.3
7	20	21	Imported	0.6
8	20	21	Razi	0.6

HI Assay. To determine the HI antibody titer in the vaccinated chickens, serum was collected from vaccinated chickens weekly and was subjected to the HI test for antibody detection. All the serum tests were performed in the virology laboratory of Razi Institute, Shiraz branch based on the methods recommended for antibody detection against avian influenza virus (H9) and Newcastle virus (V4 strain). The antibody titers were determined by HI tests using 1% chicken red blood cells according to the World Organization for Animal Health (OIE, 2009). The HI titers were expressed as the \log_2 of the inversion of the highest serum dilution that could inhibit the haemagglutination of red blood cells completely.

Vaccine Control. The viscosity of the vaccines was measured using a viscometer and pH of the vaccines was measured. The vaccines were evaluated for the possible microbial and fungal contamination using TIO, TSB, and blood agar media for 14 days according to the quality control protocols in the Quality Control Department (OIE, 2009)

Statistical Analysis. All the statistical analyses were carried out using IBM SPSS software version 21 statistical package for Microsoft Windows. The statistical significance of the data was assessed by one-way analysis of variance (ANOVA) and the means were compared utilizing the Duncan test. P-value < 0.05 was considered statistically significant.

RESULTS

The mean Newcastle and influenza HI titer were summed up in 15 weeks in the five vaccinated groups. According to the findings of this study, the ND/AI inactivated vaccine at the dose of 0.3 ml can provide the highest safety up to seven weeks after injection. Moreover, our study showed that oil emulsion Razi vaccine had an acceptable titer against NDV until the ninth week after the injection and reduced after that, which had started from the fifth week in imported (0.3 ml/bird) and Razi (0.2 ml/bird) vaccinated groups. HI antibody titers against AIV increased rapidly to 5.3, 5.2, and 5.4 \log_2 at two weeks post-vaccination with Razi vaccine dose of 0.3 ml/bird, Razi vaccine dose of 0.2 ml/bird, and imported vaccine dose of 0.3 ml/bird, respectively. The titer peaked to 5.8, 5.8, and 6 \log_2 at five weeks post-vaccination, respectively. Antibody titer of more than 5 \log_2 against AIV was detected until 13 weeks after vaccination. The antibody titer augmented rapidly after the inoculation of the inactivated vaccine and the mean HI titer reached 7.5 \log_2 against NDV and 5.5 \log_2 against H9N2 antigen four weeks post-vaccination by Razi vaccine. The highest HI titer was observed 4 weeks after injection with a mean HI titer of 7.5 and 7.1 \log_2 for NDV with inactivated bivalent ND/AI Razi vaccine at the doses of 0.3 and 0.2 ml/bird, respectively (Figure 1). Moreover, until five weeks post-vaccination HI antibody titer against AIV was reported at the high level of 6 \log_2 for Razi vaccine with the dose of 0.3 ml/bird and 5.8 \log_2 for the imported vaccine.

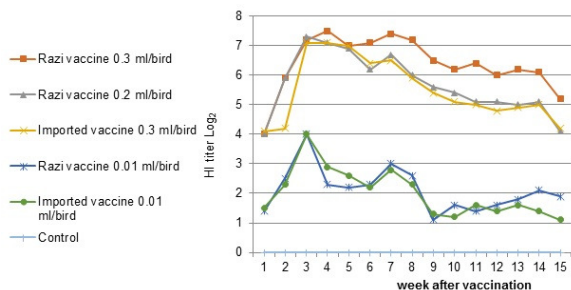


Figure 1. Comparison Newcastle antibody titer of inactivated bivalent ND and AI vaccines (Razi and imported vaccine). Three-week-old chickens were immunized by bivalent inactivated Razi and imported vaccine. The mean titers for sera collected weekly for 15 weeks after vaccination and evaluated by haemagglutination test.

The HI titers were still at a high level in 15 weeks post-vaccination with a mean HI titer of 5 \log_2 against AIV (Figure 2).

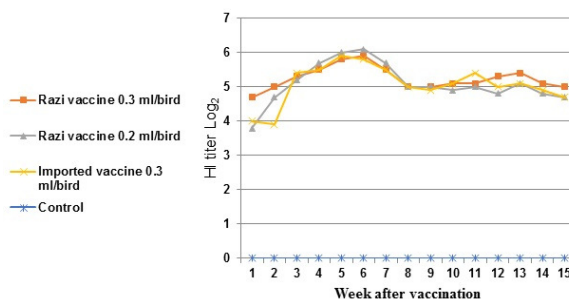


Figure 2. Comparison of influenza antibody titer between the inactivated bivalent ND and AI vaccines (Razi with 0.3ml/bird and 0.2ml/bird) three-week-old chickens were immunized by bivalent inactivated Razi and imported vaccine. The mean titers of sera collected weekly for 15 weeks post-vaccination and evaluated by haemagglutination inhibition test.

The mean Newcastle HI titer was significantly different between Razi vaccine and imported vaccine at the dose of 0.3 ml/bird ($P < 0.05$). According to the results of independent samples t-test, the difference between Razi vaccine groups with a dose of 0.3 ml and the groups that received the imported vaccine at the same dose were significantly different regarding the AI titer ($P < 0.05$). It should be noted that none of the chickens showed antibody titer against NDV and AIV pre-vaccination (Figures 1 and 2). In terms of bacterial and fungal contamination evaluation, no bacterial or fungal contamination was observed in the samples vaccinated by Razi and imported vaccines 14 days after the test. The viscosity of the vaccine samples was evaluated using a viscometer and the results for both vaccines were in the normal range. Investigations revealed no tissue reaction at the site of injection 21 days after injection with a full dose of Iranian and imported vaccines. The results of independent sample-test demonstrated that the difference between Razi and imported vaccine groups with the same dose of 0.3 ml was significant ($P < 0.05$).

DISCUSSION

Vaccination is an effective way of preventing and controlling the spread of H9N2 AIVs and NDV (Ren et

al., 2015). Some countries, such as Iran, Pakistan, and China have been using inactivated vaccines for AIV H9N2 and NDV (Naeem et al., 1999; Li et al., 2005; Banet-Noach et al., 2007). Most vaccines significantly decrease the amount of viral shedding. The rate of shedding depends on the immunity of the host, level of antibody against NDV or AIV, in addition to the dose and type of ND or AI vaccines (Miller et al., 2013). Therefore, choosing the most influential vaccine with high immunogenicity and adequate dose for prevention from the disease in chicken farms is of remarkable importance. The assessment of oil emulsion NDV and AIV vaccine immunogenicity is based on HI antibody titers. The findings of this study revealed that the antibody titer against NDV began to diminish from the seventh week after Razi vaccine injection at the dose of 0.3ml/bird. Razi institute vaccine is injected as 0.2 ml based on the commercial recommendation label, whereas it seems that it should be used at the dose of 0.3 ml in laying hens due to the long duration of their maintenance or if the high antibody titer would be recommended for longer period of time. However, the manufacturer recommended the dose of the imported vaccine as 0.3 ml, which was used accordingly in the present study. The results of independent samples t-test showed that the difference between Razi vaccine groups at a dose of 0.3 ml and the imported ones at the same dose was significant ($P < 0.05$) regarding the NDV titer. As could be observed in Table 1 and Figure 1, NDV antibody titer in Iranian vaccines is higher than the imported vaccines (Chart 1). This difference could be attributed to the greater amount of Newcastle antigens in the vaccine. The results of independent samples t-test indicated that the difference between Razi vaccine groups at a dose of 0.3 ml and the imported vaccine groups at the same dose was significant in terms of the AIV titer ($P < 0.05$). The results revealed that Razi vaccine can produce higher influenza titer, compared to the imported one. According to the literature, the inactivated oil emulsion vaccine produced by Razi Institute is effective in

reducing the spread of AIV. Hooshmand et al. (2011) examined the potency of inactivated oil emulsion vaccine produced by Razi institute for influenza concerning the circulating isolates. These authors reported that the AI vaccine decreased the spread of the virus in the vaccinated groups. According to the study carried out by Zamani Moghaddam et al. (2000), the efficacy of Razi AI vaccine was higher than the imported vaccines in producing antibody titer. Moreover, the aforementioned study showed that all groups that received Razi vaccine had lower viral shedding, in comparison with the imported vaccines. Their findings showed that the viral load was reduced in the respiratory tract of the vaccinated poultry (Zamani Moghaddam et al., 2000). As indicated in the diagrams, the antibody titer in HI test against Newcastle started to raise from the first week post-vaccination and reached a maximum of 7.2 \log_2 in 56 days for the domestic vaccine and 5.4 \log_2 for the imported one. The difference between the mean antibody titer induced by the domestic and imported vaccines might be due to the higher stimulation of the immune system by domestic vaccines. The latter point could be attributed to the amount of antigen used in the vaccine. Among the factors involved in elevating the antibody level in vaccinated chickens, the antigen strain, the amount of the antigen, and the adjuvant used in the vaccine should be mentioned (Zamani Moghaddam et al., 2000). Although it is obvious that the type and concentration of antigen in the inactivated ND and AI vaccine are the major determinants of vaccine immunogenicity, the dose of vaccine in poultry immunization is of high value (Brugh et al., 1979; Moghaddam Pour et al., 2006; Zhao et al., 2017). As a conclusion, our findings showed that the two commercial inactivated bivalent vaccines could induce an acceptable antibody response in vaccinated chickens. Nevertheless, Razi inactivated NDV/AIV vaccine at the dose of 0.3ml/bird induced a rapid and strong response in vaccinated chickens.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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