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Original Article

Comparison of the Immunogenicity of Four Infectious Bursal Disease Intermediate Vaccines in Commercial Broiler Flocks in Iran: A Field Trial Study

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ABSTRACT

Infectious bursal disease (IBD) is a highly contagious disease in young chickens worldwide. The major strategy for the prevention and control of IBD virus (IBDV) is vaccination. Therefore, the present study aimed to compare the immunogenicity of four commercially available IBD vaccines on broilers (Ross 308) that were raised in areas with very virulent IBDV infection history. Two commercial broiler farms with four standard poultry houses were selected in Alborz (n=6,250 birds per house) and Khorasan Razavi (n=8,000 birds per house) provinces of Iran. In each farm, the houses were randomly assigned to one of the four IBD intermediate vaccine brands including Dn, Vc, Ch, and Razi. The birds in Alborz were vaccinated against IBDV via drinking water at 18 and 22; and 15 and 21 days of age in Alborz and Khorasan Razavi flocks, respectively. The enzyme-linked immunosorbent assay antibody titers against IBDV were measured in 20 birds per group at 1, 28, 35, and 42 days of age. In addition, production attributes including body weight, feed conversion ratio, mortality, and production index were measured during the research period. According to the findings, the IBD antibody titers were not affected by the vaccine brands at 28, 35, and 42 days of age ($P>0.05$). Following the second IBD vaccination, an increasing trend in IBD antibody titers was noted in the Razi vaccine as well as other brands at days 35 and 42 compared to the previously recorded titers ($P<0.05$). Moreover, the production attributes of the flocks receiving various IBDV vaccine brands were not different ($P>0.05$). Regarding the productivity indices and high immunogenicity levels, the results indicated that the potential of the IBD Razi vaccine was comparable to the other investigated brands of commercial IBD vaccines, and nominated it as an immunogenic candidate vaccine for use in commercial broilers.

Keywords: Antibody titer, Broiler, Immunity, Infectious bursal disease, Vaccination

Comparaison de l'Immunogénicité de Quatre Vaccins Intermédiaires contre la Bursite Infectieuse chez les Poulets de Chair Commerciaux en Iran: une Étude de Terrain

Résumé: La bursite infectieuse est une maladie hautement contagieuse chez les jeunes poulets du monde entier. La stratégie principale pour la prévention et le contrôle du virus de la bursite infectieuse est la vaccination. Par conséquent, cette étude visait à comparer l'immunogénicité de quatre vaccins commerciaux contre la bursite infectieuse sur des poulets de chair (Ross 308) élevés dans des zones ayant des antécédents d'infection par le virus très virulent de la bursite infectieuse. Deux fermes commerciales de poulets de chair avec quatre poulaillers standard ont été sélectionnées dans les provinces iraniennes d'Alborz (n = 6,250 oiseaux par poulailler) et de Khorasan Razavi (n = 8,000 oiseaux par poulailler). Dans chaque ferme, les maisons ont été assignées au hasard

à l'une des quatre marques de vaccins intermédiaires contre la bursite infectieuse, Dans chaque ferme, les maisons ont été assignées au hasard à l'une des quatre marques de vaccins intermédiaires contre la bursite infectieuse, dont Dn, Vc, Ch et Razi. Les oiseaux d'Alborz ont été vaccinés contre le virus de la bursite infectieuse (IBDV) par l'eau potable à 18 et 22 jours; et à 15 et 21 jours dans les troupeaux d'Alborz et de Khorasan Razavi, respectivement. Les titres d'anticorps obtenus par ELISA contre le virus de la bursite infectieuse ont été mesurés chez 20 oiseaux par groupe aux jours 1, 28, 35 et 42. En outre, les attributs de production, y compris le poids corporel, le taux de conversion alimentaire, la mortalité et l'indice de production ont été mesurés pendant la période de recherche. Selon nos résultats, les titres d'anticorps contre la bursite infectieuse n'ont pas été affectés par les marques de vaccins à 28, 35 et 42 jours (P> 0,05). Après la deuxième vaccination contre la bursite infectieuse, une tendance à la hausse des titres d'anticorps contre la bursite infectieuse a été notée aux jours 35 et 42 par rapport aux titres précédemment enregistrés dans le vaccin Razi ainsi que dans d'autres marques (P <0,05). De plus, les attributs de production des troupeaux recevant diverses marques de vaccins contre le IBDV n'étaient pas différents (P> 0,05). En ce qui concerne les indices de productivité et les niveaux élevés d'immunogénicité, les résultats ont indiqué que le potentiel du vaccin de Razi contre la bursite infectieuse était comparable aux autres marques de vaccins commerciaux contre la bursite infectieuse étudiés, et représente donc un vaccin immunogène candidat à utiliser dans les poulets de chair commerciaux.

Mots-clés: Titre d'anticorps, Poulet de chair, Immunité, Bursite infectieuse, Vaccination

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious disease among young chickens worldwide. It is caused by the IBD virus (IBDV) which is a member of the family *Birnaviridae* (Murphy et al., 2000). The double-stranded RNA genome of IBDV consists of two segments, namely A and B. The larger segment A, encodes four viral proteins including two capsid proteins VP2 and VP3, the viral protease VP4 and a non-structural protein VP5. However, the smaller segment B, encodes VP1, an RNA-dependent RNA polymerase. There are informative genetic data regarding the strain variability and pathogenicity demonstrating the role of the hypervariable region within the VP2 protein (Berg, 2000; Ebrahimi et al., 2009). Based on the virulence of the serotype 1 IBDV, the etiology of the disease is classified into sub-clinical, classical virulent, and very virulent (vv) IBDV. The two major antigenic groups within serotype 1 are usually known as classical and variant. The antigenic drift is an underlying contributor to the variety of subtypes within these groups (Muller et al., 2003). Infection with IBD suppresses the immune system of birds by destroying B lymphocytes in the bursa of Fabricius and other immune organs (Tsukamoto et al., 1995). Moreover, the pathogenicity of IBDV strains involves modulating the apoptosis of chicken cells (Shahsavandi et al., 2014). The IBDV infection increases the susceptibility of the infected chickens to other viral and/or bacterial diseases and also it declines vaccination response (Mazariegos et al., 1990; Etteradossi, 2008; Lukert and Saif, 2008), which leads to considerable financial loss. To overcome such problems, the disease is often controlled by administration of attenuated or inactivated IBDV vaccines to broiler and layer flocks, especially in regions with a history of vvIBDV outbreak or in areas with epidemiological uncertainty (Palya, 1991; Rautenschlein et al., 2005; Rautenschlein et al., 2007). It must be noted that the vaccine strain and administration programs play a crucial role in the efficacy of IBDV vaccination (Mazariegos et al., 1990; Kulikova et al., 2004). Due to the immunization of broiler breeder flocks, most of the chickens received maternally-derived antibody (MDA) via the egg yolk, which resulted in their passive protection (McFerran, 1993; Lukert and Saif, 2008). Given the protection derived from MDA, replication of the virus vaccine is limited during the first weeks of their lives. According

to previous research, the vvIBDV strains may escape from MDA protection which leads to the development of clinical signs of the disease, including distress, depression, diarrhea, anorexia, ruffled feathers, trembling, and dehydration (Lukert and Saif, 2008). However, adoption of an appropriate strategy for IBD vaccination would help to overcome the problem through stimulating immune responses in chickens with moderate levels of MDA (De Wit, 2001; Mymensingh, 2002; De Wit, 2003; Block et al., 2007). Currently, the IBD has been successfully controlled by the administration of live intermediate vaccine manufactured by Razi institute (Ebrahimi et al., 2013). Protectively potential of a vaccine provides in the field and under natural challenges is the most important immunogenicity criteria of a newly manufactured vaccine. In this regard, the quality evaluation of a vaccine and comparison with foreign brands provide an unbiased assessment that is helpful to both consumers and manufacturers. Despite the widespread use of various types of vaccines for IBD in Iran, few studies have investigated the effectiveness of the IBD intermediate vaccines. Therefore, the present study aimed to compare the efficacy of several common intermediate IBDV vaccines used in commercial broilers in a field-trial study.

MATERIAL AND METHODS

General procedure. For the purposes of the study, the research was carried out in Alborz and Khorasan Razavi provinces located in the north and northeast of Iran, respectively. The farms used in this study had four standard broiler houses. Therefore, one of the four experimental IBD vaccine brands were randomly assigned to each farm. A total of 24,000 (n=6,000 birds per house) and 32,000 (n=8,000 birds per house) one-day-old broilers (Ross 308), were randomly allocated to the broiler houses located at Alborz and Khorasan Razavi, respectively. The birds in each farm were housed in commercial facilities located in areas that were at a high risk of vvIBDV. The broilers were raised under controlled conditions based on the regulations of national animal welfare. In addition to vaccination against IBDV, the birds were immunized by standard procedures against Newcastle disease, infectious bronchitis, and avian influenza as it is shown in Tables 1 and 2.

Table 1. Program of the vaccination of broiler flocks in Alborz province

Vaccine	Vaccination method	Age of vaccination (day)
ND B1, IB H120	Eye drop	1
IB 793/B	Drinking water	5
Inactivated ND + AI	Subcutaneous	10
ND B1	Eye drop	10
IBD intermediate	Drinking water	15, 21
ND B1	Drinking water	36

Table 2. Program of the vaccination of broiler flocks in Khorasan Razavi province

Vaccine	Vaccination method	Age of vaccination (day)
ND B1, IB H120	Eye drop	1
Inactivated ND + AI	Subcutaneous	7
ND B1	Eye drop	7
IB 793/B	Drinking water	14
ND LaSota	Drinking water	16
IBD intermediate	Drinking water	18, 22

Experimental design and IBD vaccination. Four commercially available intermediate attenuated IBD vaccine brands, including Razi IBD07IR (manufactured by Razi Vaccine and Serum Research Institute, Iran), Dn, Vc, and Ch were used for the vaccination of broilers in four houses in each broiler flock. Based on the recommendations of the manufacturers, the prime and second IBD vaccinations were given through drinking water. In order to determine the appropriate age for vaccination, 20 blood samples were collected from each parent flock during hatching. The time of vaccination was determined by the Deventer formula: Vaccination age={($\log_2$ IBDV antibody enzyme-linked immunosorbent assay (ELISA) titer of the bird (%)-$\log_2$ breakthrough titer of the vaccine) x t ½} + age at sampling + correcting value 0-4.

Blood sampling and serology test. Specific antibody titers against IBDV were measured by collecting blood samples from each broiler house chickens (n=20 samples per house) before the first vaccination and at 24 or 28, 35, and 42 days of age (Figures 1 and 2). After the collection of serum from blood samples, the sera were examined by an IDEXX ELISA kit (IDEXX Laboratories, Inc., Westbrook, Maine, USA) to measure specific antibodies against IBDV. Endpoint titers were calculated via the following equation: $\log_{10}$ titer=1.09 ($\log_{10}$ S/P)+3.36.

Health status and production parameters. The birds were examined daily for clinical signs of IBD and other diseases and mortality during the experimental period. The total animal loss, final body weight, feed conversion ratio (FCR), and production index were used to compare the production parameters among the evaluated IBD vaccine brands.

Statistical analysis. Before the statistical analysis, the normal distribution of data was tested using the Shapiro-Wilk test and the Univariate procedure within SAS (version 9.4). Subsequently, the data were subjected to analysis of variance by the GLM procedure of SAS in a completely randomized design. The results were reported as mean and standard deviation (SD). Tukey's test was also used for multiple comparisons of the mean and a p-value of less than 0.05 was considered statistically significant.

RESULTS

Antibody ELISA titers. Figure 1 shows the levels of antibody production against IBD during different time points of the experiment conducted in Alborz province. Mean (±SD) of MDA titer against IBDV on the first day of the experiment was 9573±20. However, it increased to 1489±23, 1360±32, 1381±25, and 1417±33 at day 28 of age by using Dn, Vc, Ch, and Razi vaccines, respectively. Following the second IBD vaccination, a significant increase in the mean antibody titers against IBDV was observed at day 35 of the experiment leading to 2.78, 2.83, 2.85, and 2.97 fold increments in the mean ELISA titers in the groups that received Dn, Vc, Ch, and Razi vaccines, compared to their corresponding values at day 24 of age, respectively. The level of the antibody also progressively increased at the end of the experiment (day 42) in all the birds that received the Razi vaccine as well as other brands (Figure 1). The highest antibody titers against IBD in vaccinated chickens were recorded at day 42 as compared to days 28 and 35 of age ($P<0.05$). Values of 5672±26, 4577±33 4643±24, and 4714±25 were recorded in the groups that received Dn, Vc, Ch, and Razi vaccines, respectively. The mean value of MDA measured on the first day of the experiment and the levels of antibody titers following IBD vaccination in the broiler flock in Khorasan Razavi are shown in Figure 2. The MDA titers were high (8410±30) in the flock located in Korasan Razavi province, which was in line with the results obtained from Alborz province. Specific IBD antibody titers at days 35 and 42 of age increased linearly, compared to the titers recorded on day 28 of the experiment (Figure 2). Results showed that the mean ELISA antibody titers against IBD were 3.28, 3.15, 3.75, and 3.22 fold higher in the groups receiving Dn, Vc, Ch, and Razi on day 35, compared to their corresponding values on day 28 of the experiment, respectively. The increasing trend was also continued until the day 42 of the experiment, where the mean ELISA antibody titers reached the highest levels and caused a 3.28, 3.79, 4.07, and 3.89 fold improvements in the groups that received Dn, Vc, Ch, and Razi, respectively, compared to the corresponding titers obtained on day 28 of the experiment.

Production attributes. The Production parameters and mortality rate of the broiler flock in Alborz province are shown in Table 3. In this study, mortality, FCR, and production index were within the range of 6.24-9.68%, 1.92-1.99, and 267-296, respectively. Production attributes were not different between the broilers receiving different IBD vaccine brands ($P>0.05$) (Table 3). The mean values of mortality, FCR, and production index in Khorasan Razavi province were within the range of 6.43-8.09%, 1.92 -1.97, and

273-292, respectively (Table 4). Therefore, the obtained results were similar in broiler flocks in both provinces.

Table 3. Health status and production parameters of broiler flocks receiving different brands of infectious bursal disease intermediate vaccine in Alborz province

Parameters	Flock No. 1 Dn vaccine	Flock No. 2 Vc vaccine	Flock No. 3 Ch vaccine	Flock No. 4 Razi vaccine
Number of the Chickens	6250	6250	6250	6250
Losses (percent)	9.68%	7.84%	7.73%	6.24%
Average weight (kg)	2.880	2.900	2.900	2.900
FCR	1.99	1.94	1.95	1.92
Production index	267	281	280	296

Table 4. Health status and production parameters of broiler flocks receiving different brands of infectious bursal disease intermediate vaccine in Khorasan Razavi province

Parameters	Flock No 1. Dn vaccine	Flock No. 2 Vc vaccine	Flock No. 3 Ch vaccine	Flock No. 4 Razi vaccine
No. Chicken	8000	8000	8000	8000
Loses (percent)	6.50%	6.43%	6.86%	8.09%
Average weight (kg)	2.935	2.873	2.908	2.867
FCR	1.92	1.94	1.93	1.97
Production index	292	282	287	273

DISCUSSION

Vaccination is one of the major tools for the prevention and control of IBDV in the poultry industry. In the present study, the immunogenicity of four commercially available vaccines was tested on the broilers raised in the area with a history of vvIBDV infection. Interference with the MDA is the major problem in the way of the administration of the attenuated IBDV vaccines in commercial broilers. Since the live vaccines are neutralized by the high levels of circulating MDA, they may not be effective during the first post-hatching days. Therefore, the efficacy of the IBD vaccination program strictly depended on the level of MDA at the time of vaccination. The importance of vaccination at the optimal time-point has been widely emphasized by the previous field and experimental studies (Rautenschlein et al., 2003; Smith et al., 2015; Smialek et al., 2016). Due to the critical role of MDA in the protection of chickens from IBD infection in the current study, the average antibody titer of the chickens was measured. The MDA titers of one-day-old chickens were within the range of 8140-9573. The ELISA titers of MDA in one-day-old chickens were divided into three levels of low (<3,000), intermediate (3,000-5,000), and high (>6,000) (De Wit, 2001, 2003). In the present study, the average MDAs at the time of hatching were recognized to be high, showing the appropriate passive immunity inherited from parent flocks. Vaccination at the inappropriate age is probably affected by MDA and causes the partial neutralization of the vaccine virus before the stimulation of the immune system of the vaccinated chickens (Tsukamoto et al., 1995; Zorman Rojs et al., 2011). Therefore, measuring MDA titer is a critical step for managing vaccination schedules. In the present study, the proper age for the IBD vaccination was calculated using the Deventer formula and was estimated at 15-18 days after they hatched. It is worth noting that under field circumstances, the MDA heterogeneity commonly seen in chickens may lead to vaccination at a very early age. Therefore, two IBD vaccinations are often advised for the achievement of a more uniform immunity (Kulikova et al., 2004; Jakka et al., 2014). Based on the previous studies, MDA could decline to an unprotective level at 15-20 days old chickens (Mymensingh, 2002). The low level of antibody titer against IBDV detected in the day 28 of the present research indicated that the MDAs had been declined to the unprotecting levels. However, following the prime and second IBD vaccinations, active immunity was developed at 42 days of age. These findings supported the selected vaccination age and the importance of second IBD vaccination in broilers. However, serological results obtained from the ELISA test showed an increase in the specific IBD antibodies in the flock vaccinated with Razi and other IBD vaccines. The mean titers obtained from broilers

vaccinated by various IBD vaccine brands were the same during the sampling time points in both locations. However, there was an increasing trend in the mean ELISA antibody titers of each group during the experimental period, which resulted in 2.8 to 4 fold increments at the end of the experiment, compared to that of day 28. Antibodies are of utmost importance in protection against IBDV (Muller et al., 2012). In the current study, the overall serological response to the IBD vaccine administration was satisfactory and resulted in the production of uniform antibody levels. The mortality rate and the average body masses measured in this study were within the standards for the Ross 308 broilers. Moreover, there were no clinical signs of disease or any symptoms associated with the vaccination. Based on these findings, the IBD humoral immune response and production attributes were the same in the birds that received Razi IBD07IR or the other brands of the vaccine.

In conclusion, the immune responses induced by the

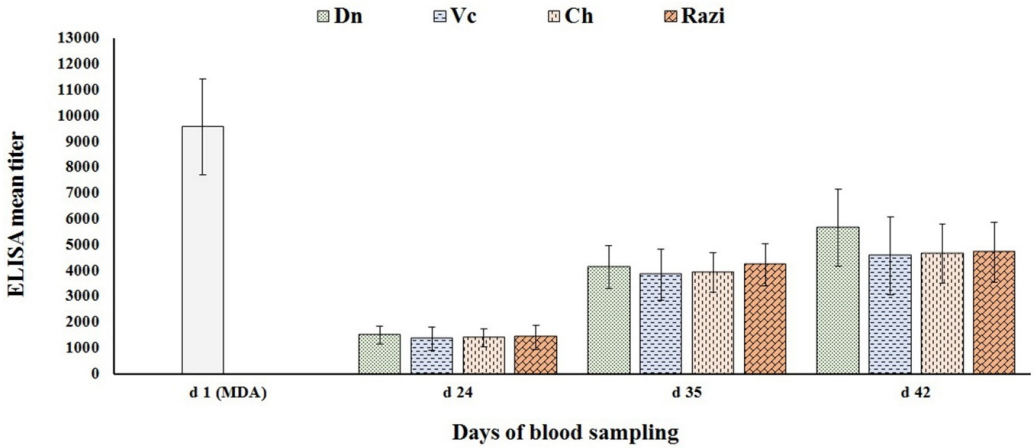

Figure 1. The ELISA antibody titers of chickens vaccinated with different vaccine brands of intermediate infectious bursal disease (Dn, Vc, Ch, and Razi) in broiler flocks in Alborz province. Note: The birds were vaccinated against infectious bursal disease virus via drinking water at 18 and 22 days of age; MDA: maternally-derived antibody; Error bar=Standard deviation.

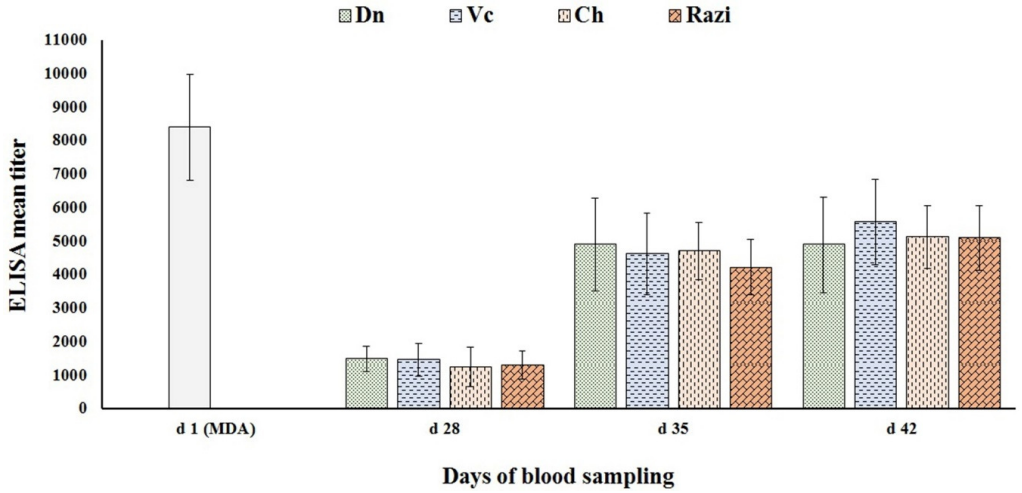

Figure 2. The ELISA antibody titers of chickens vaccinated with different vaccine brands of intermediate infectious bursal disease (Dn, Vc, Ch, and Razi) in broiler flocks in Khorasan Razavi province. Note: The birds were vaccinated against infectious bursal disease virus via drinking water at 15 and 21 days of age; MDA: maternally-derived antibody; Error bar=Standard deviation.

intermediate IBD Razi IBD07IR vaccine were comparable with the other studied brands. Moreover, the post-vaccination reactions and production attributes of flocks vaccinated with different IBD brands were similar. According to the ELISA kit producer guide (IDEXX), sera samples with values of ≥396 for IBD antibody titer are considered as positive. Therefore, the produced levels of IBD specific antibody titers by Razi IBD vaccine were sufficiently high to be considered protective. Finally, it can be concluded that the Razi IBD vaccine is an immunogenic vaccine for use in commercial broilers.

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.

Authors' Contribution

Study concept and design: Ebrahimi, M.M.

Acquisition of data: Zaghari, M., Basami, M.R.

Analysis and interpretation of data: Ebrahimi, M.M.; Yousefi, A.R.; Shahsavandi, S.

Drafting of the manuscript: Shahsavandi, S.; Ebrahimi, M.M.

Critical revision of the manuscript for important intellectual content: Ebrahimi, M.M.; Shahsavandi, S.; Yousefi, A.R.

Statistical analysis: Yousefi, A.R.

Administrative, technical, and material support: Ebrahimi, M.M.; Zaghari, M.; Basami, M.R.

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