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Original Article

Effectiveness of Thermostable Vaccine for Newcastle Disease Produced by the Razi Institute on Backyard Poultry in Iran during 2015

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ABSTRACT

Newcastle disease causes many economic losses to the poultry industry in most countries. This disease is endemic in Iran. Backyard poultry is considered the reservoir of Newcastle virus; however, there is either no vaccination program against Newcastle, or it is performed in a restricted manner. Commercial live vaccines are inactive and sensitive to temperature; moreover, vaccine delivery to villages and remote areas requires special equipment and high cost to maintain the cold chain. This study evaluated the effectiveness of a thermostable Newcastle vaccine produced by the Razi Institute (ND.TR.IR) on the backyard poultry. In four provinces, at least 4 villages were selected as the treatment group, and the same number was selected as the control group. At least, 30 birds were sampled in each village. In each group, blood samples were collected before vaccination and 2 weeks later, and the serum titer of the samples was examined with the haemagglutination inhibition test. The arithmetic mean and standard deviation of the sample titers at the rural level were compared using paired t-test before and after vaccination in each group. Moreover, Repeated Measures ANOVA was utilized to compare the vaccinated and control groups in terms of the titer changes before and after vaccination. In this study, 584 and 389 samples were taken from the treatment (53 households in 20 villages) and control groups (33 households in 14 villages). The mean serum titer values of Newcastle were 4.51±3.03 and 6.64±2.48 in the treatment group before and after vaccination, respectively ($P<0.001$). The increase in mean titer of the treatment group (2.31 log) was statistically higher than that in the control group (0.66 log) ($P<0.001$). Out of 584 birds, 517 (88.5%) ones had titer above 3 in the second turn in the treatment group. The thermostable vaccine (ND.TR.IR) produced by the Razi institute is suitable for backyard poultry, which immunizes them against Newcastle disease. Appropriate vaccination programs for backyard poultry should be made; moreover, vaccination of backyard poultry can be effective in preventing the circulation of the field viruses.

Keywords: Backyard poultry, Effectiveness, Haemagglutination inhibition (HI), ND.TR.IR vaccine, Newcastle disease

Evaluation de l'Efficacité d'un Vaccin Thermostable contre la Maladie de Newcastle Produit par l'Institut Razi sur la volaille de Basse-cour en Iran durant 2015

Résumé: La maladie de Newcastle a un impact considérable sur l'économie de l'industrie aviaire dans la plupart des pays. Cette maladie est endémique en Iran et la volaille de basse-cour est considérée comme le réservoir de la maladie de Newcastle. Cependant, il n'existe pas de protocol de vaccination contre cette maladie, ou il est effectué de manière restreint. Les vaccins vivants commerciaux sont souvent inactifs et sensibles à la

température. De plus, la livraison des vaccins dans les villages et les zones reculées nécessite un équipement spécial et un coût élevé pour maintenir la chaîne du froid. Cette étude a évalué l'efficacité d'un vaccin thermostable de la maladie de Newcastle produit par l'Institut Razi (ND.TR.IR) sur la volaille de basse-cour. Dans quatre provinces, au moins quatre villages ont été sélectionnés comme groupe de traitement et le même nombre a été sélectionné comme groupe témoin. Dans chaque village, au moins 30 oiseaux ont été échantillonnés. Dans chaque groupe, des échantillons de sang ont été prélevés avant la vaccination. Le titre sérique des échantillons a été examiné par test d'inhibition de l'hémagglutination, deux semaines plus tard. Les titres moyens arithmétiques et l'écart type des échantillons au niveau rural ont été comparés à l'aide d'un test t pour échantillons appariés avant et après la vaccination dans chaque groupe. De plus, l'analyse de variance à mesures répétées a été utilisée pour comparer les groupes vaccinés et témoins en termes de changements de titre avant et après la vaccination. Dans cette étude, 584 et 389 échantillons ont été respectivement prélevés dans les groupes de traitement (53 ménages dans 20 villages) et témoins (33 ménages dans 14 villages). Les moyennes du titre sérique spécifique au Newcastle étaient respectivement de 4.51 ± 3.03 et 6.64 ± 2.48 dans le groupe de traitement avant et après la vaccination (P<0.001). L'augmentation du titre moyen du groupe de traitement (2.31 log) était statistiquement plus élevée que celle du groupe témoin (0.66 log) (P<0.001). Sur 584 oiseaux, 517 (88.5%) oiseaux avaient un titre supérieur à 3 au deuxième tour dans le groupe de traitement. Le vaccin thermostable (ND.TR.IR) produit par l'institut Razi convient aux volailles de basse-cour et les immunisent contre la maladie de Newcastle. Des programmes de vaccination appropriés devraient être mis en place pour les volailles de basse-cour; de plus, la vaccination des volailles de basse-cour peut être efficace pour empêcher la circulation des virus d'élevage.

Mots-clés: Volailles de Basse-cour, Efficacité, Inhibition de L'hémagglutination (IHA), Vaccin ND.TR.IR, Maladie de Newcastle

INTRODUCTION

Newcastle disease virus or avian *paramyxovirus type* 1 is a member of the genus *Avulavirus* of the family *Paramyxoviridae* (Saif et al., 2008). Newcastle disease is one of the most important contagious diseases in the poultry and causes many economic losses to the poultry industry in the majority of the countries worldwide (Alexander, 1995). Many species of birds, including backyard poultry, are susceptible to the virus. The disease is endemic in some countries, including Iran, and despite widespread vaccination in industrial poultry, this disease is one of the most important challenges in poultry breeding in endemic areas (Abdoshah et al., 2012; Hosseini et al., 2014). In backyard poultry, economic issues are not important and despite the fact that Newcastle will reduce production and even causes mortality in this poultry, the poultry keepers do not pay attention to it. One of the most important challenges in industrial poultry breeding in Iran is backyard poultry and their prevalent diseases. On the one hand, many industrial farms have been located near the villages, and the workers of these fields travel to the villages. On the other hand, in most villages, backyard poultry is considered as Newcastle reservoir, and there is no vaccination program against Newcastle, or it is done in a restricted manner. Backyard poultry is kept in most Iranian villages (in more than 60000 villages) with the aim of producing meat and eggs (Mehrabadi et al., 2016). According to the Poultry Disease Database of Iran veterinary organization, about 50 million backyard poultries are kept and bred in Iran with the highest density in three Northern provinces (i.e., Gilan, Mazandaran, and Golestan). Vaccination of backyard poultry has limitations. Killed vaccines should be applied to each bird, and backyard poultries are often scattered; additionally, it is difficult and time-consuming to catch each of them. Therefore, this type of vaccine is not a good option for vaccination of backyard poultry. On

the other hand, commercial live vaccines are inactive and sensitive to temperature (Bell et al., 1995), and vaccine delivery to villages and remote areas requires special equipment and high cost to maintain the cold chain. That is why the use of Newcastle vaccines produced by apathogenic thermostable strains is advantageous in rural areas and can be a good alternative. The two strains of V4 and I2 are used to produce NDV-thermostable vaccines; however, strain I-2 is more resistant than V4 (Alders, 2014). The ND I-2 vaccine produced by the Australian Centre for International Agricultural Research that is suitable for local production of thermostable vaccines without cost to laboratories in developing countries (Bensink and Spradbrow, 1999; Adwar and Lukesova, 2008). This study evaluated the effectiveness of a thermostable Newcastle disease vaccine (ND.TR.IR) produced by the Razi vaccine and serum research institute on the backyard poultry in Iran.

MATERIAL AND METHODS

Studied Regions and Statistical Population. This randomized controlled trial was conducted in Khorasan Razavi, Bushehr, Khuzestan, and Kurdistan provinces during October and December 2015. These provinces were chosen due to the prevalence of Newcastle disease in these regions based on the poultry industry report; moreover, they were willing to cooperate with the implementation of the project. In each province, at least 4 villages were selected as the treatment group, and the same number was selected as the control group. It should be noted that Kurdistan province had only a treatment group. At least 30 birds were sampled in each village (Alders and Spradbrow, 2001). A list of villages was first prepared, and subsequently, eight villages with no history of vaccination against Newcastle disease during the last 6 months were selected randomly from each province using Excel software. The villages (n=8) were randomly assigned into two groups of treatment and control. The treatment groups were subjected to vaccines, whereas the control groups received no vaccines. In all the villages in these provinces, the vaccination was performed completely; however, the sampling was carried out merely in the selected villages and households.

Vaccination. The vaccine used in this study is a thermostable one produced by the Razi vaccine and serum research institute with a brand name of ND.TR.IR. A 500-dose vial was dissolved in 10 liters of cool water without foreign objects, detergents, and disinfectants. Following that, 20 cc (containing 1 dose) of the solution was allocated to each bird, and the water containing the vaccine for all birds of a household was poured in a clean plastic container without disinfectants or detergents. Subsequently, it was taken by the birds immediately. Before vaccination, the birds were not allowed to drink water for three hours.

Sampling and Tests. In each group, blood samples were collected before vaccination and 2 weeks after vaccination. In the second stage, samples were taken from households that were sampled in the first stage; however, the same birds were not sampled necessarily. In addition, if a new bird had arrived within two weeks, the sampling would not have performed, and samples were taken from birds that were present in the first stage in the household. In case of multiple species in a household, samples were taken from all species and all age groups. At each sampling, at least 1 mL of blood was taken from the brachial vein. The blood samples were placed at room temperature for one h. The sera were then transferred to the laboratory and were stored at -20° C. All tests were performed at the Central Laboratory of the Iran Veterinary Organization.

Vaccine Effectiveness. Regarding the evaluation of the vaccine effectiveness, the serum titer of the samples was examined before and after vaccination with the haemagglutination inhibition (HI) test (OIE, 2017) at two levels of birds and villages. In total, a 4-unit B1 antigen produced by the Razi vaccine and serum research institute was used to carry out the HI tests. Serum dilutions were done based on log2, and 1% red

blood cell was prepared from specific-pathogen-free chickens.

Data Analysis. The data were analyzed in SPSS software (version 22). In addition, the arithmetic mean±SD of the titers of the samples at the rural level were compared before and after vaccination in each group using a paired t-test. Moreover, repeated-measures ANOVA was utilized to compare the vaccinated and control groups regarding titer changes before and after vaccination. A p-value less than 0.05 was considered statistically significant.

RESULTS

The samples were taken from 584 birds in the treatment group (53 households in 20 villages) and 389 birds from the control group (33 households in 14 villages). The mean serum titers of Newcastle were 4.51±3.03 and 6.64±2.48 in the treatment group before and after vaccination, respectively (P<0.001). Moreover, the mean serum titers of Newcastle disease were 4.44±2.59 and 5.10±2.40 in the control group in the first and second turns, respectively (P<0.05). The increase in the mean titer of the treatment group (2.31 log) was statistically higher than that in the control group (0.66 log) (P<0.001) (Figure 1).

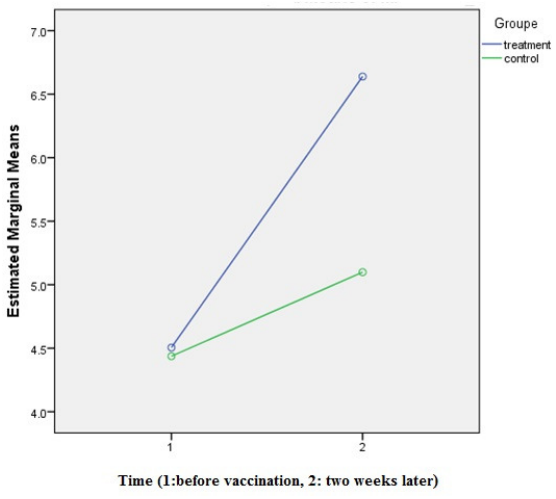

Figure 1. Mean titer of Newcastle disease in treatment (ND.TR.IR) and control groups before vaccination and two weeks later in backyard poultry in Iran during 2015.

In the provinces of Khuzestan, Bushehr, Khorasan Razavi, and Kurdistan, the mean serum titers in the treatment groups significantly increased two weeks after vaccination (P<0.001). Furthermore, the maximum and minimum titer values were observed with 2.92 log and 1.31 log in the provinces of Khorasan Razavi and Bushehr, respectively. Table 1 summarizes the sera results in both groups. Out of 584 birds in the treatment group, 517 (88.5%) samples had titer above 3 in the second turn. The mean titer and coefficient of variation of the villages were 58.21% and 31.8% in the vaccinated group before and after vaccination, respectively (P<0.001). Moreover, the corresponding values were 58.86% and 36.21% in the control group, respectively (P<0.05). The mean titer and coefficient of variation in the vaccinated group were significantly lower in the second time, compared to the control group (P<0.05). During the study period, none of the villages showed mortalities due to Newcastle disease.

DISCUSSION

According to the studies, all velogenic strains of Newcastle disease viruses have been isolated from backyard poultry. Velogenic strains are endemic in these birds, and annual outbreaks of the disease will be possible in rural poultry in endemic areas. The spread of the virus is slow due to the low exposure and scattering of the backyard poultry, and it is transmitted by the oral-fecal route. In rural poultry, there are several factors involved in the outbreaks, such as bird immunity status, the age groups, the pathotype of the virus, race, comorbidity, season, multiple heterogeneous strains, and no bird enclosure (Awan et al., 1994). Infected birds are the main source of infection and freshly recovered birds are acting as the reservoir of the disease. Although few birds will recover from this disease, they will be virus carriers for a short time. Therefore, rural poultry, as a reservoir, causes Newcastle virus to survive and circulates in the region (Alders and Spradbrow, 2001). On the other hand, one of the most important aspects of backyard poultry

vaccination is the dynamics of poultry, as well as the production and reproduction of backyard poultry in each region. The reproduction and breeding of backyard poultry lead to a new susceptible population whose production times and population size are very important in the selection and frequency of vaccinations. In a study conducted in Cameroon during 1992, on average, 12 hens and 3 roosters were kept per household. Moreover, there were three production cycles per hen, and 3-5 chickens were produced per cycle yearly. In total, 144 chickens were produced per 15 hens in one year (Bell et al., 1995). In the present study, the mean serum titer against Newcastle increased by 2 logs two weeks after vaccination in the treatment group. However, the mean titer increased by about 0.66 logs two weeks later in the control group. In similar studies in other countries, the mean serum titer increased by 5 log three weeks after vaccination, and the highest titers following vaccination were observed 2-4 weeks after vaccination (Bell et al., 1995). In an experimental study performed in Nigeria, the HI titers of the vaccinated birds with the I-2 vaccine increased two weeks after the second vaccination and ranged from 0.0 log2 to 8.7 log. The mean HI titer peaked; however, it declined prior to challenge at nine weeks of age (Abah et al., 2017). The level of serum titer was high in most of the villages sampled before vaccination indicating the circulation of the virus among backyard poultry in most villages of Iran. Moreover, a low increase in the serum titer observed in the control group can be attributed to two reasons. First, samples in the second time were taken from the same birds in the first stage; however, they may not be exactly the same birds. As a result, different titers can be due to variations in the blood-sampled birds. The other reason for the increase in the titers of the control group can be the continuous circulation of the virus in backyard poultry and their constant exposure to circulating viruses. The increase in the mean titer of the treatment group was different in the studied provinces; therefore, the highest increase of titer was 3 log in Khorasan Razavi province. This difference in titer increase may be due to the presence of the antibody in the birds during vaccination, which affects the efficiency of the vaccine (Dimitrov et al., 2017). As shown in the present study, the mean titer increase after vaccination was lower in the villages in which the mean titer was higher before vaccination. Another interesting point in the present study is that vaccination led to the reduced coefficient of variation of titer and its uniformity in vaccinated birds in addition to an increase in the mean titer. This suggests that the vaccinated population is less likely to be infected by a virus, thereby minimizing the spreading of the virus. The results of an experimental study conducted by Asl Najjari et al. on broiler chicken in Iran showed that vaccination by I-2 vaccine could protect the birds against mortality and also decreased virus shedding. Moreover, there were no significant

Table 1. Mean titer of Newcastle disease in treatment (ND.TR>IR. vaccine) and control groups before vaccination and two weeks later in backyard poultry in Iran during 2015

Province	Group	Number of sampled villages	Number of sampled families	Number of sampled birds	Mean titer (village level)			P-Value
					Before vaccination	2 weeks later	Differences	
Kurdistan	Treatment	6	18	172	5.28±3.48	7.15±2.60	1.86	<0.001
Khuzestan		5	12	141	5.11± 2.30	7.07±2.15	1.95	< 0.001
Bushehr		3	8	93	4.95±2.81	5.92±1.92	1.13	<0.001
Khorasan Razavi		6	15	178	2.81±2.65	5.73±2.77	2.92	<0.001
Total		20	53	584	4.51±3.03	6.64±2.48	2.31	<0.001
Khorasan Razavi	Control	5	13	152	3.63±2.53	4.41±2.85	0.73	<0.001
Bushehr		4	8	93	5.29±2.46	5.06±1.94	- 0.23	0.326
Khuzestan		5	12	144	4.62 ±2.54	79.5±1.60	1.97	< 0.001
Total		14	33	389	4.44±2.59	5.10±2.40	0.66	< 0.05

differences between vaccination with I-2 and B1 vaccines. They concluded that vaccination with thermostable I-2 could be a proper candidate to campaign against ND in tropical areas of Iran (Najjari et al., 2017). According to the results of a study, 88.5% of the poultry in the vaccinated group had a titer of 3 or higher, as protecting titers, two weeks after vaccination (Van Boven et al., 2008). This increased titer has been different in various studies. In a study performed by Jagne et al. (1991) on vaccination with food, 30% of the birds had a titer higher than 3 four weeks after vaccination, whereas in a study carried out by Bell et al. (1995), all vaccinated birds had titer of three or higher by eye drop vaccination three weeks after treatment. The differences in titer amount can be due to the vaccination method, such as eye drop vaccination, which is usually more effective (Bell et al., 1995). Based on the obtained results, the employed vaccine not only increased the mean titer of the vaccinated poultry but also improved the herd immunity by more than 85%. For Newcastle viruses, the herd immunity above 85% can prevent the disease to become epidemic (Van Borm et al., 2005), and this level of immunity has been obtained in this study. The thermostable vaccines induce protective immunity in backyard poultry if correctly applied. Additionally, they are cheap, and therefore, affordable to all farmers to use; moreover, they are easy to administer by farmers without requiring strict cold chain facilities (Adwar and Lukesova, 2008). Based on the results, the thermostable vaccine (ND.TR.IR) produced by the Razi vaccine and serum research institute is suitable for backyard poultry, which immunizes them against Newcastle disease. Regarding the conditions of backyard poultry breeding in Iran, there are probably continuous production and reproduction of backyard poultry in the villages, the continuous introducing of susceptible birds, the existence of different species of birds with different ages, and the contact of susceptible birds with wild birds in some parts of the country. On the other hand, due to the favorable environmental conditions and heterogeneity of viruses in the country, the aforementioned factors will keep Newcastle disease viruses in backyard poultry. Therefore, concerning different dynamics and the process of production and breeding of backyard poultry in Iran, appropriate studies ought to be carried out on the appropriate vaccination dates and the number of vaccinations based on different conditions governing the backyard poultry breeding system in different regions of the country. Accordingly, appropriate vaccination programs should be made for backyard poultry; moreover, vaccination of backyard poultry can be effective in preventing the circulation of the field viruses.

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 conflicts of interest regarding the publication of the study.

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