Archives of Razi Institute, Vol. 75, No. 1 (2020) 31-37

DOI: 10.22092/ARI.2019.122830.1228

Original Article

Efficacy of Thermostable Newcastle Disease Virus Strain I-2 in Broiler Chickens Challenged with Highly Virulent Newcastle Virus

Habibi [1]*, H., Firouzi [2], S., Nili [2], H., Asasi [2], K., Mosleh [2], N.

1. Department of Animal Sciences, Agricultural and Natural Resources College, Persian Gulf University, Bushehr, Iran
2. Avian Diseases Research Center, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Received 18 August 2018; Accepted 03 February 2019
Corresponding Author: h.habibi@pgu.ac.ir

ABSTRACT

Newcastle disease (ND) is a major threat to poultry industry production throughout developing countries. The Newcastle disease viruses (NDVs) infecting industrialized and indigenous poultry in Iran are velogenic strains and responsible for the frequent outbreaks of ND in poultry farms even in vaccinated flocks causing serious economic losses in the commercial and indigenous poultry. However, vaccination is the only way to protect against endemic ND, and the conventional vaccines are not heat stable and consequently require complex cold-chains to be transferred to users leading to not much resistance. The present study aimed to evaluate the efficacy of thermostable NDV strain I-2 in broiler chickens vaccinated via drinking water and coated on oiled wheat grain. The horizontal transmission of I-2 strain and transmission of disease from vaccinated to unvaccinated chickens were also evaluated in this study. The obtained results showed that both routes of administration, following primary and/or secondary dose, provoked the production of necessary antibody titer and adequate protective immunity in broiler chickens. Moreover, the horizontal transmission of I-2 strain from vaccinated to unvaccinated chickens housed together induced an antibody response and protected unvaccinated chickens against a local field isolate of a virulent strain of NDV (The intravenous pathogenicity index 2.46, mean death time 59 h). Nevertheless, all unvaccinated and Newcastle challenged broilers chickens against the NDV died in this study. It is noteworthy that the transmission of the virus from challenged broiler chickens was very low to induce clinical signs in susceptible chickens. The obtained results of this study revealed the efficacy of NDV strain I-2 coated on the oiled wheat and via drinking water as it protects broiler chickens from highly virulent NDV.

Keywords: Newcastle disease, Thermostable strain, Strain I-2, Broiler chicken

Efficacité de la Souche Thermostable du Virus de la Maladie de Newcastle I-2 chez des Poulets de Chair Contaminés par le Virus de Newcastle Hautement Virulent

Résumé: La maladie de Newcastle (ND) constitue une menace majeure pour la production industrielle de volaille dans les pays en développement. Les virus de la maladie de Newcastle (NDV) qui infectent les volailles industrialisées et indigènes en Iran sont des souches vélogènes. De plus, ils sont responsables de fréquentes épidémies de ND dans les élevages de volailles même dans le cas des troupeaux vaccinés et ils provoquent de graves pertes économiques dans la volaille commerciale et indigène. Par conséquent, la vaccination est le seul moyen de contrôler la ND endémique. Cependant, les vaccins conventionnels ne sont pas stables à des températures élevées et nécessitent que les chaînes du froid complexes soient transférées aux utilisateurs, ce qui entraîne peu de résistance. La présente étude visait à évaluer l'efficacité de la souche thermostable NDV I-2 chez des poulets de chair vaccinés via de l'eau potable et nourris avec du grain de blé enrobé d'huile. La transmission horizontale de la souche I-2 et la transmission de maladies des poulets vaccinés aux poulets non vaccinés ont

également été évaluées dans cette étude. Les résultats de cette étude ont montré que les deux méthodes d'administration du vaccin produisaient les titres d'anticorps nécessaires et une immunité protectrice adéquate à la première dose et après la deuxième vaccination. De plus, la transmission du virus vaccinal du poulet de chair vacciné au poulet de chair non vacciné a provoqué la production d'anticorps et une protection contre NDV (IVPI 2.46, MDT 59h) chez le poulet de chair non vacciné. Cependant, tous les poulets de chair non vaccinés et contaminés par Newcastle sont morts dans cette étude. Il est à noter que la transmission du virus à partir de poulets de chair contaminés était très faible et n'induisait pas des signes cliniques chez les poulets les plus sensibles. Les résultats de cette étude ont révélé l'efficacité de l'administration via l'eau potable de la souche NDV I-2 enrobée de blé huilé, conférant ainsi une protection contre le NDV hautement virulent chez les poulets de chair.
Mots-clés: Maladie de Newcastle, Souche thermostable, Souche I-2, Poulet de chair

INTRODUCTION

Newcastle disease (ND) is a major threat to the poultry production industry throughout developing countries. The Newcastle disease viruses (NDVs) infecting industrialized and indigenous poultry in Iran are velogenic strains and responsible for the frequent outbreaks of ND in poultry farms even in vaccinated flocks causing serious economic losses in the commercial and indigenous poultry (Habibi et al., 2015). With this endemic nature of ND either in commercial or village chicken and little possibility of enforcing efficient biosecurity measures to prevent the spread from village to commercial poultry, vaccination remains the only alternative strategy for controlling and minimizing the effect of the disease (Alexander, 2001). The eradication of the virus is not feasible; therefore, vaccination programs should be continual and sustainable. Despite the plethora of vaccines and aggressive vaccination programs being practiced by the poultry industry all over the world to control the disease, ND has defied all logic and continues to rear its ugly head in both epidemic and endemic forms causing monumental economic losses in the industry. None of the usual commercial ND vaccines will partially protect chickens against the disease if the vaccines reach the chickens in a potent form. However, there are frequent outbreaks of the disease even in vaccinated flocks. Many commercial vaccines are thermolabile and sometimes extremely thermolabile. Cold chains are too expensive to maintain and develop. Heat-resistant vaccines enable distributors and users to reduce the problems associated with the inadequacy of cold chains in the field. While evaluating vaccines for their ability to protect from ND, it may be useful to include their ability to decrease virus shedding in vaccinated birds following exposure to wild field virus, which potentially reduces the spread of NDV, especially in an outbreak setting. A thermostable ND vaccine strain I-2 has been initially recommended for use in developing countries for the protection of village chickens against ND (Bensink and Spradbrow, 1999). However, the present study aimed to establish the efficiency of the thermostable variant of strain I-2 as the thermostable vaccine in broiler chickens locally produced at the Avian Diseases Research Center of Shiraz University in Iran. Three vaccination strategies were used to obtain the following results: 1) the efficacy of the I-2 vaccine in broiler chickens challenged with highly virulent virus under laboratory conditions. 2) The horizontal transmission of the I-2 vaccine virus. 3) The transmission of the disease

MATERIAL AND METHODS

Experimental design. A total of 180 commercial day-old broiler chicks (Cobb 500) of mixed gender

were purchased from a local hatchery in Fars province, Iran. All the experimental measures were conducted in the controlled laboratory condition of the Avian Diseases Research Center of Shiraz University. A total of 150 four-week-old chicks, which had been tested negative for ND antibody, were randomly allocated to experimental groups to test whether the locally produced I-2 NDV would stimulate chickens to produce a protective haemagglutination inhibition (HI) antibody titer or not. During the study, water and food were provided *ad libitum*. All birds were fed on a standard commercial diet based on corn and soybean meal. Strict sanitation practices were maintained in the unit before and during the course of the experiment. Sixty chicks identified with individual wing tags were randomly placed into rooms 1, 2, 3, and 4 (15 chicks in each room). Rooms 5, 6, and 7 were kept as the positive control, vaccine control, and negative control groups, respectively (Table 1). Birds in rooms 1, 2, and 6 were vaccinated by means of drinking water, and the birds in rooms 3 and 4 were vaccinated with food containing vaccines. The chickens (in rooms 1, 2, 3, 4, and 6) were vaccinated on day 28. The chickens in rooms 2 and 4 were revaccinated 1 week later (day 35).

Transmission study. To evaluate the horizontal transmission of the I-2 vaccine virus, 2 h after the termination of vaccination, eight unvaccinated chickens were kept in contact with the vaccinated chickens of the rooms 1, 2, 3, and 4 throughout the experimental period. To assess the transmission of the disease, 2 h after the challenge (day 42) of the vaccinated and in-contact chickens, seven sensitive chickens (seronegative to ND virus) were added to the rooms 1, 2, 3, and 4.

I-2 strain. The strain I-2 of NDV was propagated in 9-day-old embryonated fowl eggs from the vaccine master seed produced and supplied by the Department of Veterinary Pathology of the University of Queensland, Australia (Spradbrow and Copland, 1996). The vaccine harvested from embryonated eggs was reported with a titer of $10^{10.2}$ EID50/ml.

Coating carrier food. The wheat grain was cracked to small sizes to ease swallowing by the birds. Then, it was sieved for the removal of dust content. To remove the effects of some inactivating and inhibitory substances on the wheat grain (e.g., acids and lectins), it was autoclaved at 121°C for 20 min, and 0.3 kg of cracked wheat was mixed with 30 mL of blended 10% glycerol before mixing with the vaccine. Thirty doses of vaccine (each dose equal to $10^{6.5}$ EID50/bird) from the allantoic fluid of the strain I-2 virus were reconstituted in 30 ml of phosphate-buffered saline and thoroughly mixed with a wheat carrier. After mixing, the coated food vaccine was spread on trays and kept at room temperature to be dried for 3 h. The virus content of the dried coated wheat was estimated to be $10^{5.5}$ EID50/g.

Vaccination. A 10 g of the coated food vaccine ($10^{5.5}$ EID50/g), equal to a dose for one chicken, was presented to each bird. For vaccination via drinking water, a 40 dose vial of NDV I2 strain was reconstituted in 1 liter of distilled water, and each bird was given 25 ml of the reconstituted vaccine. Each dose contained $10^{6.5}$ EID50/ml ND I-2 virus.

Challenge trail. A very virulent field isolate of the velogenic NDV was selected as the challenge virus previously characterized and registered in GeneBank by the name of JF820294.1. The embryo infective dose (EID50) was calculated according to the Reed and Muench (Habibi et al., 2015) formula and reported as $10^{8.3}$ EID50/ml. The intravenous pathogenicity index (IVPI) of the challenge virus was 2.46, and the mean death time of chicken embryos was 59 h. Birds were intranasally challenged with 0.05 ml allantoic fluid containing $10^{4.3}$ EID50/ml at day 42.

Serology. Sera samples for subjecting to the HI test were collected to determine the antibody titers of NDV according to the method of Allan and Gough (Allan and Gough, 1976). The ND HI antigen was manufactured by Razi Vaccine and Serum Research Institute in Iran. The haemagglutination (HA) titer was diluted to contain 4 HA units for the use in the HI test.

Clinical observation and statistical analysis. The birds were daily monitored after the challenge for clinical signs, and on days 5, 10, and 17 after the challenge, two chickens from each group were humanely euthanized by cervical dislocation and examined for the presence of gross lesions. Morbidity, mortality, and protection rates for each group were calculated in the study. The data of the mean titers of the immune response to the NDV were analyzed using one-way analysis of variance by SPSS software (version 16.0).

Table 1. Experimental design for evaluating efficacy of thermostable Newcastle disease vaccine strain I-2 in broiler chickens challenged with highly virulent virus

Room	Group		Birds (n)	Challenge (day 42)
1	1	Vaccinated[a] via drinking water	15	+
	2	In contact[b] with vaccinated chickens of group 1	8	+
	3	In contact with chickens of group 1 and 2 after challenge	7	-
2	4	Vaccinated twice[c] via drinking water	15	+
	5	In contact with vaccinated chickens of group 4	8	+
	6	In contact with chickens of groups 4 and 5 after challenge	7	-
3	7	Vaccinated via food containing vaccine	15	+
	8	In contact with vaccinated chickens of group 7	8	+
	9	In contact with chickens of groups 7 and 8 after challenge	7	-
4	10	Vaccinated twice via food containing vaccine	15	+
	11	In contact with vaccinated chickens of group 10	8	+
	12	In contact with chickens of groups 10 and 11 after challenge	7	-
5	13	Positive control	10	+
6	14	Vaccine control (vaccinated via drinking water)	10	-
7	15	Negative control	10	-

[a] Primary vaccination was performed on day 28.
[b] In-contact chickens were not vaccinated and were seronegative to Newcastle disease virus.
[c] Secondary vaccination was performed on day 35.

Significant differences among different groups were identified at 5% by Duncan's multiple range test.

RESULTS

The HI titer of maternal antibody in one-day-old chicks was $\log_2$ 5.9. Before vaccination, all chickens were seronegative to the NDV antibody (HI Geometric Mean Titer [GMT] log2<1). The HI antibody response to different vaccination protocols is shown in Table 2. On day 42 (14 days after the primary vaccination and/or on the day of the challenge), 100% of the chickens in the vaccinated groups and even of in-contact chickens had a HI titer above $\log_2$ 3. The birds of group 4 (which were vaccinated via drinking water and revaccinated 1 week later) had the highest HI antibody titer with the mean titer of $\log_2$ 4.8 among vaccinated groups ($P<0.05$). In addition, 17 days after the challenge, the highest HI antibody mean titers were reported for groups 4 and 10 (Table 2; $P<0.05$). All the vaccinated and in-contact birds appeared healthy after the challenge and did not show any clinical signs of ND until the end of the experiment (17 days following the challenge). Furthermore, the sensitive birds added to rooms 1, 2, 3, and 4 to evaluate the transmission of the disease did not show any signs of the disease; however, they serologically became positive for NDV (Table 2). In this study, 100% protection and no gross lesion were observed in necropsy among the groups of rooms 1, 2, 3, and 4 that were humanely euthanized. On the other hand, 100% of positive control birds showed clinical signs, including dyspnea, nasal discharge, anorexia, green diarrhea, tremor, as well as torticollis, and finally, all of them died up to day 11. In the necropsy of the birds in the positive control group, petechial hemorrhages in the proventriculus, cecal tonsils, hyperemia, and exudates in the trachea and lungs were observed.

DISCUSSION

Vaccination as a means of protecting birds against ND is routinely practiced around the world. Despite the extensive use of vaccines, the outbreaks of ND have

still been recorded in Asia and Middle East that might be due to the genetic or antigenic variation in vaccine and circulating viruses, inadequacy of vaccines, or no correct application of vaccines (Shabbir et al., 2013).

vaccine demonstrated a more uniform response and consequently higher GMT values in comparison to the maize coated vaccine.

Although the lower limit of the recommended

Table 2. Antibody titer (Log_2) tested by haemagglutination inhibition assay of broiler chickens vaccinated with Newcastle disease virus I-2 vaccine administered through drinking water and food carrier vaccine and in-contact birds

Room	Group	1 week after primary vaccination	2 weeks after primary vaccination[a]	Challenge (day 42)	17 days after challenge	Protection rate (%)[b]
	1	1.9±0.8	4.2±0.8	+	6.75±0.8	100
Drinking water containing vaccine (1x)	2	1.3±0.5	4±0.5	+	6.75±1.1	100
	3	0.75±0.5	0.5±0.5	-	5±1.8	100
	4	2±1.0	4.8±1.0	+	7.66±1.0	100
Drinking water containing vaccine (2x)	5	1.2±0.8	4.4±0.8	+	6.5±1.2	100
	6	0.75±0.5	0.5±0.5	-	4.8±2.0	100
	7	1.6±0.7	3.5±0.7	+	6.75±0.8	100
Food containing vaccine (1x)	8	1.1±0.7	3±0.7	+	6±0.9	100
	9	0.75±0.5	0.5±0.5	-	5.5±1.3	100
	10	1.5±0.5	4±0.5	+	7.33±1.2	100
Food (2x)	11	1.3±0.8	3.25±0.8	+	6.25±1.6	100
	12	0.75±0.5	0.5±0.5	-	5±1.9	100
Positive control	13	0.75±0.5	0.5±0.5	+	-[c]	0
Vaccine control	14	1.3±0.7	4.4±0.5	-	4.75±0.5	-
Negative control	15	0.75±0.5	0.5±0.5	-	0.5±0.6	-

[a] Secondary vaccination in groups 4 and 10 was performed 1 week after the primary vaccination.
[b] No clinical signs and death were considered 100% protection.
[c] All birds died up to day 11 after the challenge.

In the present study, it was tried to investigate, under controlled laboratory conditions, the efficacy of thermostable ND virus strain I-2 in broiler chickens. This experiment provided the information on antibody responses and adequate protection in broiler chickens, where the birds vaccinated twice via drinking water with a one-week interval produced higher HI titer values (Table 2). However, all groups showed 100% protection against overt clinical signs and mortality upon the challenge with highly virulent NDV (IVPI 2.46). The production of higher HI antibody titer by means of drinking water vaccination than that by food coated vaccine was also previously shown in several studies (Habibi et al., 2015; Abdi et al., 2016; Lawal et al., 2016). It was indicated that the direct oral drench

protective antibody level measured by HI test for ND vaccines in intensively reared commercial chickens is 4 based on Log_2 (Sarcheshmei et al., 2016), it has been reported that HI antibody titer of Log_2 3 was considered to be adequate for food-based vaccines administered orally to scavenging chickens (Echeonwu et al., 2007). The resistance of the chickens even with the HI antibody titer of $\log_2 \leq 4$ challenged with the velogenic ND virus in the present study indicated that other immune system components could possibly contribute to the immunity against ND. This finding suggested a mechanism for reducing the amount of virus shedding in addition to the antibodies measured in HI assays. Several studies with orally administered ND vaccines showed that there was an increased number of secreting

immunoglobulin A (IgA) referred to as secretory antibodies on the mucosal surfaces of the avian intestine, bronchi, and oviduct (Jayawardane and Spradbrow, 1995). The measurement of mucosal IgA and cellular immune responses in future studies with these recombinants may provide direct evidence of the aforementioned finding. Due to close contact between birds when they are housed together, the lateral transmission of the live vaccine virus is possible (van Boven et al., 2008). The obtained results of the present experimental study demonstrated that nonvaccinated birds in the same room (housed together with vaccinated birds) did not show any mortality following the challenge with a very virulent field strain. The horizontal spread of thermostable live NDV can result in the production of protective HI antibody, which was also previously reported (Habibi et al., 2015). It has been shown that the vaccination of susceptible birds against ND and avian influenza protects birds against more serious consequences of the disease; however, virus replication and shedding may still occur, albeit at a reduced level (Costa-Hurtado et al., 2015). In the present study, susceptible in-contact birds exposed to challenged birds became seropositive and did not show any clinical signs. This finding implies the inability of the I-2 strain virus for the full protection against viral replication and possible shedding of challenge virus but reduced viral shedding to induce disease in susceptible birds. The reduction of viral shedding from vaccinated birds infected with NDV could potentially minimize the impact of an outbreak and help to prevent the spread of the disease. Based on the previous recommendations regarding the vaccination of village chickens using food-based vaccines, the multiple administration of the vaccine is needed (Spradbrow and Samuel, 1991; Wambura et al., 2000; Habibi et al., 2015). In the present study on broiler chickens, even the primary dose of food-based I-2 strain virus has been protective. The results of this study showed that wheat coated with ND I-2 strain was able to protect susceptible broiler chickens against the challenge with a very pathogenic field isolate of NDV (JF820294.1) in comparison to the control group. Previous studies showed that using parboiled sorghum and parboiled sorghum coated with gum has not been protective in local and commercial chickens (Lawal et al., 2016), and uneven results from previous studies have been obtained in this regard (Habibi et al., 2015). Antiviral factors constituent in the seed or introduced as preservatives have been reported to be responsible for insufficient protection in previous studies (Echeonwu et al., 2007).

The basic principles for ND prevention are similar to those reported for other diseases, including good management practice and biosecurity or good hygiene in conjunction with vaccination. In the present experimental study, it was shown that the administration of I-2 vaccines via drinking water and/or coated on the wheat grain was safe and immunogenic and provoked the production of protective antibody response following the vaccination of broiler chickens. The successful implementation of field trials depends on good laboratory-field communications. Pretrial planning and extension are crucial to ensure farmer cooperation in these trials and any future vaccination programs.

Ethics

All animal studies were approved by the Ethics Committee of Shiraz University and performed in accordance with the ethical standards set in the 1964 Declaration of Helsinki and its later amendments.

Conflict of Interest

None of the authors have financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of the present study.

Authors' Contribution

Study concept and design: H Nili and K Asasi
Acquisition of data: H Habibi and S Firouzi
Analysis and interpretation of data: N Mosleh

Drafting of the manuscript: H Habibi and S Firouzi
Critical revision of the manuscript for important intellectual content: H Nili, H Habibi and S Firouzi
Statistical analysis: N Mosleh
Administrative, technical, and material support: H Habibi and S Firouzi

Acknowledgment

The authors are grateful to Professor Peter Spradbrow affiliated to the Department of Veterinary Pathology at the University of Queensland, Australia, for the supply of the seed vaccine of the NDV I-2 strain. The authors would also like to thank Dr. Hadi Roholahizadeh for valuable technical assistance.

References

Abdi, R.D., Amsalu, K., Merera, O., Asfaw, Y., Gelaye, E., Yami, M., *et al.*, 2016. Serological response and protection level evaluation in chickens exposed to grains coated with I2 Newcastle disease virus for effective oral vaccination of village chickens. BMC Vet Res 12, 150.

Alexander, D.J., 2001. Gordon memorial lecture. Newcastle disease. Br Poult Sci 42, 5-22.

Allan, W.H., Gough, R.E., 1976. A comparison between the haemagglutination inhibition and complement fixation tests for Newcastle disease. Res Vet Sci 20, 101-103.

Bensink, Z., Spradbrow, P., 1999. Newcastle disease virus strain I2–a prospective thermostable vaccine for use in developing countries. Vet Microbiol 68, 131-139.

Costa-Hurtado, M., Afonso, C.L., Miller, P.J., Shepherd, E., Cha, R.M., Smith, D., *et al.*, 2015. Previous infection with virulent strains of Newcastle disease virus reduces highly pathogenic avian influenza virus replication, disease, and mortality in chickens. Vet Res 46, 97.

Echeonwu, G., Iroegbu, C., Echeonwu, B., Ngene, A., Olabode, A., Okeke, O., *et al.*, 2007. Delivery of thermostable Newcastle disease (ND) vaccine to chickens with broken millet grains as the vehicle. Afr J Biotechnol 6, 2694-2699.

Habibi, H., Nili, H., Asasi, K., Mosleh, N., Firouzi, S., Mohammadi, M., 2015. Efficacy and transmissibility of Newcastle disease I-2 vaccine strain against a field isolate of virulent ND virus (JF820294.1) in village chicken. Trop Anim Health Prod 47, 73-78.

Jayawardane, G.W., Spradbrow, P.B., 1995. Mucosal immunity in chickens vaccinated with the V4 strain of Newcastle disease virus. Vet Microbiol 46, 69-77.

Lawal, J., El-Yuguda, A., Ibrahim, U., 2016. Efficacy of feed coated Newcastle disease i2 vaccine in village chickens in Gombe State, Nigeria. J Vet Sci Technol 7, 349.

Sarcheshmei, M., Dadras, H., Mosleh, N., Mehrabanpour, M., 2016. Comparative evaluation of the protective efficacy of different vaccination programs against a virulent field strain of the Newcastle Disease virus in broilers. Braz J Poul Sci 18, 363-370.

Shabbir, M.Z., Zohari, S., Yaqub, T., Nazir, J., Shabbir, M.A., Mukhtar, N., *et al.*, 2013. Genetic diversity of Newcastle disease virus in Pakistan: a countrywide perspective. Virol J 10, 170.

Spradbrow, P., Copland, J., 1996. Production of thermostable Newcastle disease vaccines in developing countries. Prev Vet Med 2, 157-159.

Spradbrow, P.B., Samuel, J.L., 1991. Oral Newcastle disease vaccination with V4 virus in chickens: comparison with other routes. Aust Vet J 68, 114-115.

van Boven, M., Bouma, A., Fabri, T.H., Katsma, E., Hartog, L., Koch, G., 2008. Herd immunity to Newcastle disease virus in poultry by vaccination. Avian Pathol 37, 1-5.

Wambura, P., Kapaga, A., Hyera, J., 2000. Experimental trials with a thermostable Newcastle disease virus (strain I2) in commercial and village chickens in Tanzania. Prev Vet Med 43, 75-83.