

# Thermoresistant Newcastle disease vaccine effectively protects SPF, native, and commercial chickens in challenge with virulent virus

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## Abstract

**Background:** Due to the more stability and a better homogeneity in immune response, the use of thermoresistant vaccines in different chicken types has been increased.

**Objective:** This study aimed to evaluate the efficacy of a newly developed Newcastle disease vaccine (ND.TR.IR) originating from I-2 strain in specific pathogen-free (SPF) and native and broiler chickens.

**Methods:** Following determination of pathogenicity indices on the candidate seed, three efficacy examinations were conducted. In the first experiment, 120 1-day-old SPF chickens were randomly allocated to six groups and either vaccinated with ND.TR.IR via eye drop at 1, 7, and 21 days of age ( $V_1$ ,  $V_7$ , and  $V_{21}$ ), or considered as non-vaccinated control groups ( $C_1$ ,  $C_7$ , and  $C_{21}$ ). At 20th post-vaccination day, sera hemagglutination inhibition (HI) antibody titres against ND virus (NDV) were measured and then the chickens were challenged by virulent NDV (vNDV). In the second and third experiments, the efficacy of ND.TR.IR vaccine was compared to routine vaccination program (B1 and LaSota) in native and broiler chickens that were vaccinated at 10 and 20 days of age, respectively. The HI antibody titres were measured on 10, 20, 30, and 40 days of age, and also challenge efficacy test with vNDV was conducted on 30 days of age.

**Results:** The studied virus, as a vaccinal seed, complied with the pathogenicity indices of avirulent NDV and molecular identity of I-2 strain. In the efficacy evaluation trials, the vaccinated chickens had higher HI antibody titres against NDV compared with their corresponding control chickens ( $p < 0.05$ ). Results of the challenge tests indicated 95% and 100% protection against vNDV in native, SPF, and broiler-vaccinated chickens, respectively.

**Conclusions:** The present findings indicated that administration of ND.TR.IR induced appropriate HI antibody titres against NDV in SPF, native, and broiler chickens associated with good protection in efficacy test.

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## KEYWORDS

chicken, efficacy, Newcastle disease, thermoresistant, vaccine

## 1 | INTRODUCTION

Newcastle disease (ND), a highly contagious and widespread poultry disease, is an underlying contributor to economic loss in commercial and backyard poultry flocks (Lawal et al., 2016). The rural poultry farms play a pivotal role in food chain supply in developing countries; however, they have often been affected by infectious diseases such as ND, occurring by avian paramyxovirus-1 (Kattenbelt et al., 2006; Shahid Yousafzai & Liaquat-Ahmad, 2017). Restriction of virulent ND viruses (vNDV) circulation in the backyard chickens could decrease the risk of the virus transmission to commercial farms (Adwar & Lukesova, 2008). Vaccination against ND is the most feasible and protective prevention approach against ND outbreaks since the 1940s (Dias et al., 2001). Because of a relatively high cost and requiring long times to induce immunity, as well as the need to be injected by trained people, the use of inactivated ND vaccines is limited in village flocks. The routine live vaccines (B1 and LaSota) are cheaper and easier to administrate, which have made them more acceptable to be administrated in rural conditions. Nevertheless, maintenance of the cold chain for live vaccines is the main concern to achieve effective ND vaccination in faraway areas (Ahlers et al., 1999; Adwar & Lukesova, 2008).

In developing countries, complying with standard storage and cold chain are somehow difficult that resulted in a considerable reduction of vaccine efficacy. Approximately, a 2-h timeline for administration of the dissolved and prepared common ND vaccines is regarded as a major limitation on their administration in rural flocks. Although lyophilization and stabilizing excipients have conferred marginal stability on many vaccines, the lack of vaccines that possess adequate thermostability is often a primary barrier to global vaccination efforts (Rexroad et al., 2002).

Under inappropriate conditions of the cold chain, administration of a thermoresistant ND vaccine could be the primary strategy (Adwar & Lukesova, 2008). Several thermoresistance viruses driven from I-2 or HR-V4 strains have been introduced to manufacture ND live vaccines (Adwar & Lukesova, 2008). It has been shown that the efficacy of heat-resistant ND vaccines is kept up to 2 months at 9–29°C, and 2 weeks at 30–37°C (Alders, 2014). In this regard, previous investigations (Fentie et al., 2014; Hassanzadeh et al., 2020; Nasser et al., 2000; Van Boven et al., 2008) also showed a high protection against vNDV in chickens vaccinated by I-2.

There are many village chickens and other backyard poultry that are also potential reservoirs for threatening of commercial farms to NDV (Hassanzadeh & Bozorgmeri Fard, 2004). In this regard, the prevention of ND occurrence at village flocks is a crucial step in maintenance sustainable agriculture and control of ND outbreaks in the poultry industry. Most of the investigations on thermostable ND vaccines have focused on their usage in rural flocks; however, their efficacy on commercial chickens is rarely studied. Therefore, this study was aimed

to evaluate the efficacy of a developing Newcastle disease I-2 vaccine (ND.TR.IR) against ND in specific pathogen-free (SPF) and native chickens as well as commercial broilers.

## 2 | MATERIALS AND METHODS

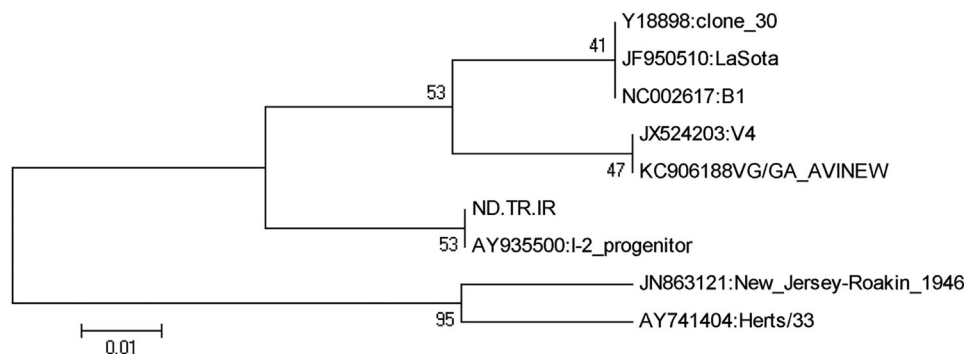
### 2.1 | Molecular identification of vaccinal virus and bioinformatics analysis

A triplicate molecular analysis of the F gene sequences and their corresponding amino acids were carried out to determine the identity of the virus. Virus RNA was extracted using a high pure viral Nucleic Acid kit (Roche, Germany) according to the manufacturer's instructions. The extracted RNA was used to synthesize cDNA using the RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific). An 840 bp segment of the F gene sequence including cleavage site was amplified using forward, 3'-CATCTTCTACCAGGATCCA-5', and reverse, 3'-CCAAGAGTTGAGTCTGTGAGTC-5' primers (Frozandeh-Moghadam et al., 2009). Polymerase chain reaction (PCR) was performed using a 5 µl cDNA aliquot in 50 µl; final volume, including 10 µl PCR buffer (10X) consisting of 1.5 mM MgCl<sub>2</sub>, 1 µl 10 mM dNTPs, 1 µl of 10 pM of each primer, 1 µl (2 U) Taq DNA polymerase, and 31 µl double distilled water. Following initial denaturation at 94°C for 4 min, 35 cycles including denaturation at 94°C for 60 s, annealing at 50°C for 60 s, and extension at 72°C for 8 s were performed using a standard thermocycler (Eppendorf-Netheler-Hinz GmbH, 5331). The amplicon was then electrophoresed in 1% low melting point agarose and visualized under UV light using a transilluminator (biostep.de, GmbH). Thereafter, the 840 pb bands were sliced and purified using a high pure PCR product purification kit (Roche) and sequenced using the PCR primers and Comfort Read method (Bioneer Co., Korea).

The nucleotide sequences were edited and trimmed and then were translated to F protein amino acid sequence of the virus. The amino acid sequences were then aligned and compared with commercial vaccinal (B1, LaSota and its clone, VG/GA, V4, I-2) and standard vNDV (Roakin, Herts33) strains using BioEdit V7.0.0 software. A phylogenetic analysis was also carried out to identify evolutionary relationships among the studied virus and some virulent and vaccinal NDV strains using MEGA 6.0 software with Neighbour-Joining algorithm and 1000 Bootstraps (Figure 1).

### 2.2 | Pathogenicity indices

To measure pathogenicity indices, fresh infective allantoamniotic fluid (AAF) with haemagglutination (HA) titre >2<sup>4</sup> was diluted in sterile normal saline (W. H. Allan et al., 1978). The intracerebral pathogenicity



**FIGURE 1** The phylogenetic analysis of the ND.TR.IR F-gene and some commercial vaccines (B1, LaSota and its clone, VG/GA, V4, I-2) and standard virulent Newcastle disease virus (vNDV) (Roakin, Herts33) strains datasets, inferred using the Neighbour-Joining method

index (ICPI) was determined via intracerebral injection of 0.05 ml of the diluted virus into each of 10 1-day-old SPF chickens. The chickens were daily evaluated for general and clinical signs of ND, during the next 8 days and scored as follow: 0 for normal, 1 for sick, and 2 for dead chickens. The ICPI was calculated as the mean score/chicken/observation over the examination period. The intravenous pathogenicity index (IVPI) was determined by intravenous injection of 0.1 ml of the diluted virus into 10 6-week-old SPF chickens. Chickens were daily observed for general and local clinical signs during the next 10 days and scored at each observation. The score zero was regarded for normal, 1 for sick, 2 for paralyzed, and 3 for dead chickens. The IVPI was calculated as the mean score per bird per observation over the 10 days (W. H. Allan et al., 1978). Mean embryo death time (MDT) was determined through inoculation of 0.1 ml of freshly diluted virus from each dilution ranged  $10^{-6}$ – $10^{-9}$  into the allantoic cavity of five 9- to 10-days-old embryonated SPF chicken eggs at 8:00 AM, and incubation at 37°C. The remaining virus dilutions were kept at 4°C. Similarly, 0.1 ml of each dilution was inoculated into another five eggs at 5:00 PM and incubated at 37°C. All the incubated eggs were examined twice each day to record times of any embryo deaths during the next 7 days. The minimum lethal dosage was determined as the highest virus dilution in which all of the inoculated embryos died. The MDT was calculated as the mean time in hours for the minimum lethal dose to kill embryos (W. H. Allan et al., 1978).

## 2.3 | Serological and challenge efficacy tests

The efficacy of the ND.TR.IR was evaluated through three separate experiments in SPF, commercial broiler and backyard chickens. All the challenge tests were undertaken by intramuscular injection of  $\geq 10^5$  LD<sub>50</sub> of a vNDV (Herts33) at biosafety level 3 in BioFlex B40 Rigid Body Poultry Isolator (Bell Isolation Systems Ltd, UK).

### 2.3.1 | Efficacy evaluation in SPF chicken

A total of 120 newly hatched SPF chickens were randomly allocated to three vaccinated (V1, V7, and V21) and three control (C<sub>1</sub>, C<sub>7</sub>, and C<sub>21</sub>) groups (20 chickens/group). The vaccinated chickens were inocu-

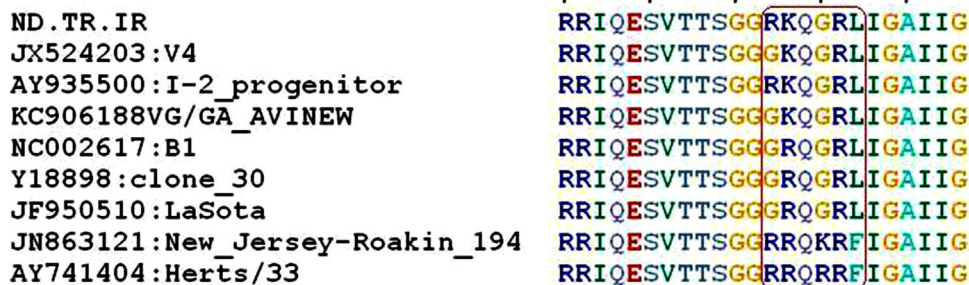
lated with  $\leq 10^6$  EID<sub>50</sub> of the vaccinal virus (ND.TR.IR) through the eye drop at 1 (V1), 7 (V7), and 21 (V21) days of age. Meanwhile, the controls chickens received distilled water by eye drop. Twenty days after each vaccination time, all of the vaccinated and non-vaccinated chickens were challenged. Blood samples were taken from the experimental groups to measure hemagglutination inhibition (HI) antibody titres against NDV immediately before the challenge test. During the next 10 days, daily mortality was recorded, and the survived chickens were controlled for general clinical signs of ND (OIE, 2012).

### 2.3.2 | Efficacy evaluation in native chicken

A total of 320 1-day-old native chickens were reared during 50 days of the experimental period. Maternally derived antibody (MDA) against NDV was determined on 20 randomly selected chickens on 1 and 10 days of age (10 chickens/sampling times). The sampled chickens were removed from the trial, and the remaining chickens were randomly allotted into three experimental groups (100 chickens/group) including B1+LaSota group: inoculation of commercial B1 and LaSota vaccines, at 10 and 20 days of age, respectively; ND.TR.IR group: inoculation of ND.TR.IR vaccine at 10 and 20 days of age and Control group: no vaccine administration. Vaccine inoculation at 10 and 20 days of age was conducted through eye drop and drinking water, respectively. To measure HI antibody titres against NDV, blood samples were collected (10 chickens/group) at 20, 30, and 40 days of age. On day 30 of age, 20 chickens per group were transferred to isolators and challenged. [Correction added on 8 April 2022, after first online publication: “10 chickens” was corrected to “20 chickens” in the preceding sentence] During the next 10 days, daily mortality was recorded, and the survived chickens were checked for general clinical signs of ND.

### 2.3.3 | Efficacy evaluation in broiler chicken

To evaluate the efficacy of ND.TR.IR in broiler chickens, a total of 470 1-day-old Ross 308 broiler chickens (20 chickens for determining MDA, and 450 experimental chickens [ $n = 150$  chickens /group]) were reared under standard environmental and nutritional conditions according to Ross Broiler Management Handbook. Except for the number of



**FIGURE 2** Comparison of the amino acid sequence deduced from the F-gene nucleotide sequence of ND.TR.IR virus with several commercial vaccinal (B1, LaSota and its clone, VG/GA, V4, I-2) viruses and virulent Newcastle disease virus (vNDV) (Roakin, Herts33) strains datasets. The ND.TR.IR amino acid sequence is accessible under AHH29550.1. Note: the motifs located in the box representing ND.TR.IR and other NDVs F-protein cleavage site

challenged chickens which was 10 per group, all of the procedures were as same as Section 2.3.2 (OIE, 2012).

## 2.4 | Data analysis

Data obtained from sera HI antibody titres were statistically analyzed using a generalized linear model (GLM) procedure of SAS, 9.4 (SAS, 2004). Before the analysis, the normal distribution of the data was tested by the Shapiro–Wilk test and UNIVARIATE procedure. Mortality rate, as binary distributed data, was analyzed by GENMOD procedure using a logit odds ratio link function. The mathematical model was as follows:  $Y_{ij} = \mu + T_i + e_{ij}$  where  $Y_{ij}$  is observations,  $T_i$  is a treatment effect, and  $e_{ij}$  is residual random error. Results were reported as mean  $\pm$  standard error (SE). Tukey's test was used for multiple comparisons, and statistical differences were declared at  $p < 0.05$ .

## 3 | RESULTS

### 3.1 | Virus identity

Phylogenetic analysis was based on fusion (F) protein gene of ND.TR.IR virus, and some commercial vaccines and standard vNDV strains are represented in Figure 1. The sequence analysis demonstrated a complete identity (100%) between ND.TR.IR and I-2 strain, resulting in distribution at the same cluster. The F gene nucleotide sequence of the studied virus is available at NCBI gene bank as ND.TR.IR under the accession number KF805771.1.

Amino acid sequence comparison of the studied virus with some commercial vaccinal virus and standard vNDV strains is shown in Figure 2. The studied virus showed a complete consistency with I-2 strain, while it had considerable F-protein amino acid sequence differences compared to other NDV strains. The proteolytic cleavage site of ND.TR.IR is characterized by three basic amino acids at the carboxy terminus of F2 and leucine at the amino terminus of F1 (<sup>112</sup>R-K-Q-G-R\*<sup>117</sup>) associated with the avirulent NDVs. [Correction added on 8 April 2022, after first online publication: “five basic amino acids” was corrected to “three basic amino acids” in the preceding sentence]

**TABLE 1** In vivo pathogenicity indices of ND.TR.IR and specifications of vaccinal Newcastle disease virus (NDV) strains

Pathogenicity indices	Standard acceptable value*	Result
ICPI	<0.5	0.0
IVPI	0.0	0.0
MDT (h)	>90	138

Abbreviations: ICPI, intracerebral pathogenicity index; IVPI, intravenous pathogenicity index; MDT, mean death time.

\*Values representing specifications of vaccinal NDV strains.

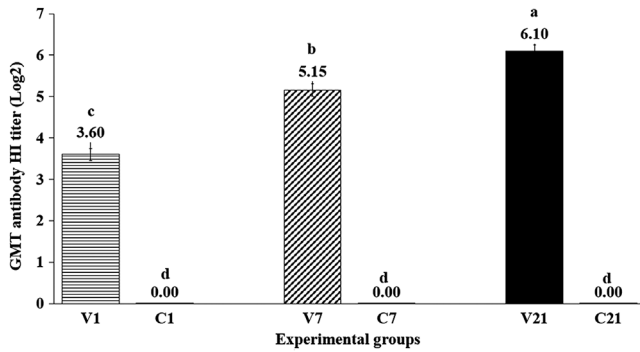
### 3.2 | Pathogenicity indices

Results of conventional in vivo pathogenicity tests and specifications of vaccinal NDV strains are presented in Table 1. Both ICPI and IVPI values measured for ND.TR.IR were zero. The mean death time (MDT) was 138 h.

### 3.3 | Serological and challenge efficacy results

Antibody titres against NDV in SPF chickens that were inoculated with ND.TR.IR are shown in Figure 3. Vaccination increased mean HI antibody titres in V1, V7, and V21 groups compared to their corresponding control groups ( $p < 0.05$ ). It is worth noting that vaccination in elder chickens caused higher antibody titres than that of youngers ( $p < 0.05$ ). Results of the challenge test showed that ND.TR.IR administration caused 100% protection in the vaccinated chickens compared to their corresponding control chickens.

Mean HI antibody titres against NDV in native chickens immunized via B1 and Lasota or ND.TR.IR vaccines are represented in Figure 4. The B1+LaSota and ND.TR.IR groups had no significant difference during the experimental period; however, they had higher HI antibody titres compared to the control chickens ( $p < 0.05$ ). The MDA titre was decreased from first ( $7.2 \pm 0.24$ ) to tenth ( $5.6 \pm 0.26$ ) days of the experiment. The decreasing trend in MDA was continued in the control group and reached  $2.7 \pm 0.24$ ,  $2.0 \pm 0.21$  and  $<1$  at 20, 30, and 40 days of age,



**FIGURE 3** Hemagglutination inhibition (HI) antibody titres against Newcastle disease virus in specific pathogen free chickens vaccinated by the I-2 strain (ND.TR.IR) at different ages. Note: Chickens were vaccinated with a dose of ND.TR.IR (V1, V7, and V21) or received distilled water (C<sub>1</sub>, C<sub>7</sub>, and C<sub>21</sub>) through eye drop at 1, 7, and 21 days of age. Antibody titres were measured 21 days after each administration time. Values with different superscripts (a and b) indicate a significant difference ( $p < 0.05$ ). Error bar = standard error of the mean

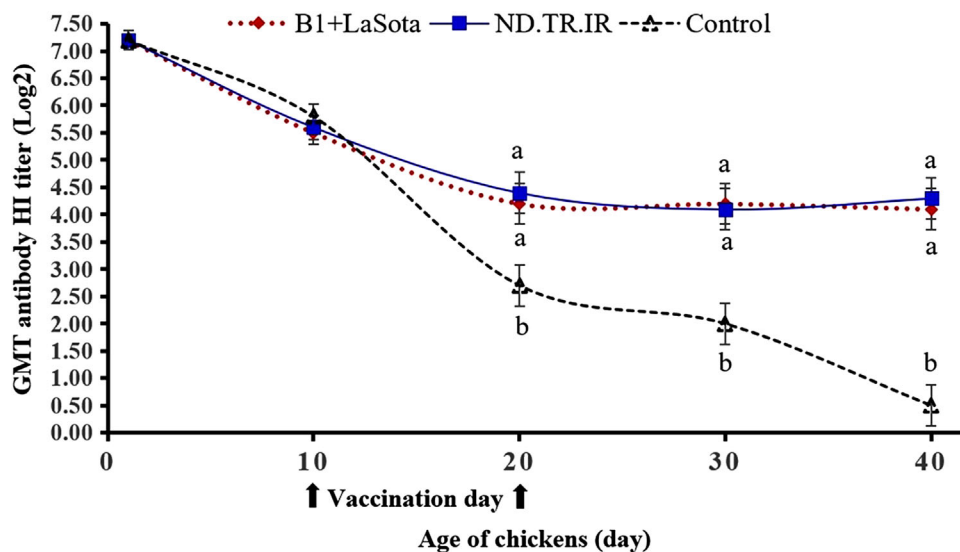
respectively. Mean HI antibody titres in the B1+LaSota and ND.TR.IR groups showed a gradually decreasing trend between 10 and 20 days of age; however, following the booster vaccination at day 20 of age, the antibody levels remained at a constant level (ranged between  $4.1 \pm 0.36$  and  $4.4 \pm 0.42$ ). Results of the challenge test showed 95% protection in B1+LaSota and ND.TR.IR groups which was higher than 35% of the control group ( $p < 0.05$ ; Table 2).

Mean HI antibody titres against NDV in broilers inoculated with different ND vaccines are represented in Figure 5. The antibody levels were not different between B1+LaSota and ND.TR.IR groups over the experimental period ( $p < 0.05$ ), but they had significantly higher HI

antibody titres compared to the control chickens ( $p < 0.05$ ). A severe decreasing trend in MDA titres was noted from first ( $5.8 \pm 0.18$ ) to tenth ( $1.7 \pm 0.22$ ) days of the experiment. However, following the first ND vaccination, the antibody levels were increased in B1+LaSota ( $4.30 \pm 0.45$ ) and ND.TR.IR ( $4.90 \pm 0.27$ ) groups compared to the control chickens on day 20 of age ( $p < 0.05$ ). Thereafter, the antibodies titre of B1+LaSota and ND.TR.IR groups showed a relatively constant trend and reach  $4.8 \pm 0.41$  and  $4.7 \pm 0.42$  on day 30, and  $4.3 \pm 0.49$  and  $4.2 \pm 0.39$  on day 40 of age, respectively. Results of the challenge test in this experiment showed full protection (100%) in both B1+LaSota and ND.TR.IR vaccinated chickens, while all the unvaccinated challenged chickens (control) died (Table 2).

## 4 | DISCUSSION

The welling for administration thermoresistant vaccines, especially in village condition, is increasing during last decades. The I-2 is the main ND strain used for producing thermoresistant ND vaccines for rural chickens. However, its potential use in emergency vaccination and no evidence for reverts to virulence has also made it a candidate vaccine to control ND in commercial flocks (Bisschop et al., 2021). In an attempt to produce a thermoresistant ND vaccine, in this study, an I-2 originated vaccinal seed was successfully characterized and its efficacy against ND was investigated in SPF, native, and broiler chickens. Results of the present study revealed a complete identity between genomic sequences of the studied virus and I-2 strain showing the potential of the studied virus as a vaccinal seed. According to W. H. Allan et al. (1978), an avirulent NDV complies with the IVPI, ICPI, and MDT indices when their corresponding values are zero, less than 0.5, and longer than 90 h, respectively. Consistent with the molecular



**FIGURE 4** Effect of administration a thermoresistant Newcastle disease (ND) vaccine (ND.TR.IR) versus common vaccination programme (B1 and LaSota) on antibody titre against ND in native chickens. Note: The chickens received a dose of ND.TR.IR or B1 and LaSota ND vaccines at days 10 and 20 of age via eye drop and drinking water, respectively. Values with different superscripts (a and b) within each sampling time indicate a significant difference ( $p < 0.05$ ). Error bar = standard error of the mean

**TABLE 2** Protection level of specific pathogen-free (SPF), native, and broiler chickens vaccinated with common vaccination program (B1 and LaSota) or a thermoresistant Newcastle disease (ND) vaccine (ND.TR.IR) in the challenge with virulent ND virus

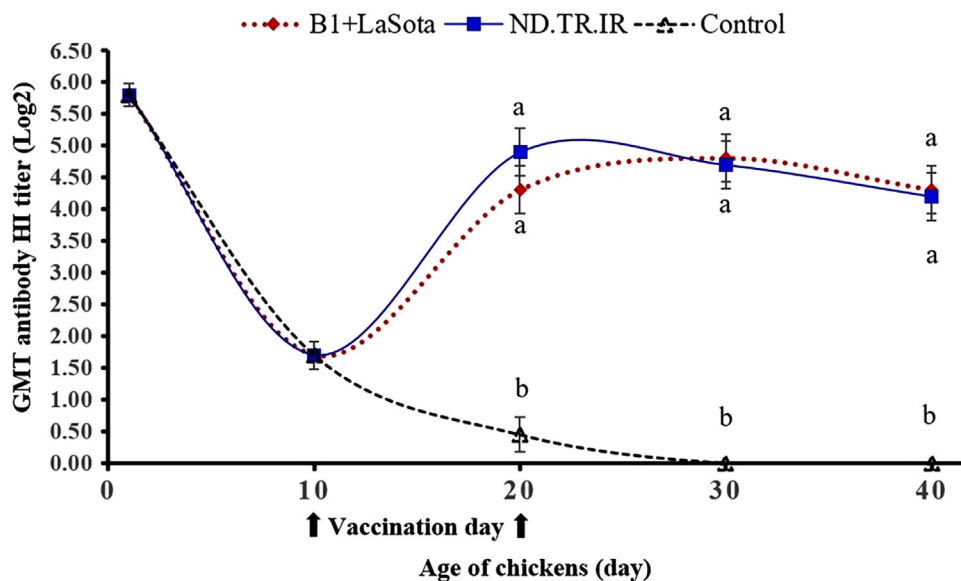
Experimental groups	V1	V7	V21	Control (C <sub>1</sub> , C <sub>7</sub> , and C <sub>21</sub> )
SPF <sup>1</sup>	100% (10/10) <sup>a</sup>	100% (10/10) <sup>a</sup>	100% (10/10) <sup>a</sup>	0% (0/10) <sup>b,2</sup>
Experimental groups	-	B1+LaSota	ND.TR.IR	Control
Broiler <sup>3</sup>	-	100% (10/10) <sup>a</sup>	100% (10/10) <sup>a</sup>	0% (0/10) <sup>b</sup>
Native chicken <sup>3</sup>	-	95% (19/20) <sup>a</sup>	95% (19/20) <sup>a</sup>	35% (7/20) <sup>b</sup>

<sup>1</sup>Chickens in V1, V7, and V21 groups were vaccinated with a dose of ND.TR.IR or received distilled water (C<sub>1</sub>, C<sub>7</sub>, and C<sub>21</sub>) through eye drop at 1, 7, and 21 days of age, respectively.

<sup>2</sup>Results of control groups in C<sub>1</sub>, C<sub>7</sub>, and C<sub>21</sub> were the same.

<sup>3</sup>The chickens received B1 and LaSota or ND.TR.IR vaccines at days 10 and 20 of age via eye drop and drinking water, respectively.

<sup>a,b</sup>Within each row, values with different superscripts are significantly different ( $p < 0.05$ ).



**FIGURE 5** Effect of administration a thermoresistant Newcastle disease (ND) vaccine (ND.TR.IR) versus common vaccination program (B1 and LaSota) on antibody titre against ND in broiler chickens. Note: The chickens received a dose of ND.TR.IR or B1 and LaSota ND vaccines at days 10 and 20 of age via eye drop and drinking water, respectively. Values with different superscripts (a and b) within each sampling time indicate a significant difference ( $p < 0.05$ ). Error bar = standard error of the mean

identification results, the obtained values for the pathogenicity indices approved that the ND.TR.IR is a candidate vaccinal seed.

In the present study, three experiments were conducted to evaluate the efficacy of ND.TR.IR. According to the OIE (2012), the efficacy evaluation of a live ND vaccine must be conducted on 10 or more SPF or other fully susceptible chickens, at the minimum recommended age using the minimum recommended dose. Meanwhile, to obtain more conclusive results, in the first experiment, the efficacy of the ND.TR.IR vaccine was examined in different ages of chickens. Our results showed that the inoculation of 1-day-old SPF chickens with ND.TR.IR was associated with sera antibody titres greater than  $2^3$ , at 21 days post-vaccination. The higher antibody titres which were observed in the chickens inoculated at 7 and 21 days of age is explained by the developed immune system in the elder chickens (Ambali et al., 2017; Al-Garib et al., 2003). The appropriate serological immune response in

chickens received ND.TR.IR was supported by the result of the challenge efficacy test, where the vaccinated chickens were fully protected against vNDV infection. It has been documented that chickens with HI antibody titers  $\geq 2^3$  would be protected following the challenge efficacy test (W. Allan & Gough, 1974; Hossain et al., 2010; Hassanzadeh et al., 2020; Van Boven et al., 2008). However, there are pieces of evidence showing that the chickens with lower titer also may survive after the challenge (Meers & Spradbrow, 2006). In addition to the circulating antibody, cellular and mucosal immunity are also involved in acquiring a good protection level in a challenge with the virulent virus; however, because of determination of circulating antibody is more feasible and easier, it is considered as the primary index for evaluation protection against diseases.

In the second experiment, the efficacy of ND.TR.IR on native chickens was evaluated and compared with a routine vaccination

program. Results showed an appropriate HI antibody raise and protection against vNDV in all of the vaccinated chickens. In agreement with 95% protection recorded in this experiment, Ilonggo et al. (2008) reported that thermostable I-2 ND vaccine provided 100% and 89% protection against ND in-housed and unhoused chickens, respectively. Moreover, investigations on rural flocks have revealed that I-2 originated vaccines were associated with approximately 80% coverage and long-term immunity (Adwar and Lukesova, 2008). Historically, thermostable ND vaccines have been developed at the Queensland University and used in several developing countries to control ND in rural flocks (Meers & Spradbrow, 2006); however, due to more stability in ambient temperature and lateral transmission, the thermostable ND vaccines are usually associated with a lower variation in antibody titres and cause better protection against ND (Alders & Spradbrow, 2001; Illango et al., 2008; Shahid Mahmood et al., 2014). These advantages have made the thermostable ND vaccine as a good option for the use in commercial broiler farms.

Results of the third experiment efficacy test showed that immune response derived from ND.TR.IR or routine ND vaccines (B1 and LaSota) was not different in the commercial broilers. Despite the negligible difference in sera antibody titres, both immunization programmes were associated with full protection against vNDV. These findings were in line with the results of the second experiment in native chickens. Following vaccination, the immunized chickens acquire humoral and cellular as well as mucosal immunity. However, by the intramuscular route of challenge, the vNDV is bypassed through mucosal immunity that is one of the first defensive barriers against pathogens. Therefore, resistance to challenge with the intramuscular route is regarded as the gold standard for evaluating the efficacy of viral vaccines (Meers & Spradbrow, 2006). It has been widely accepted that herd immunity provides some protection to suboptimal or unvaccinated chickens (Hassanzadeh & Bozorgmeri Fard, 2004; Kapczynski et al., 2013) if more than 85% of the chickens acquire HI antibody titres greater than  $2^3$  after two ND vaccinations (Van Boven et al., 2008). The current results were corroborated by the previous studies which reported that antibody titre  $\geq 2^3$  is the possible protective level in chickens vaccinated against ND (W. Allan & Gough, 1974; Bensink & Spradbrow, 1999; Wambura, 2011). Meanwhile, Kapczynski and King (2005) suggested that at commercial broiler housing, only chickens with HI titres greater than  $2^4$  survived in vNDV challenge and 66% of chickens with lower antibody titres remained as unprotected. More commonly, HI titres  $\geq 2^5$  are typically suggested as a protective level (W. H. Allan et al., 1978; Kapczynski et al., 2013). In this study, 100%, 80%, and 60% of the ND.TR.IR-inoculated broiler chickens had HI antibody titres  $\geq 2^3$ ,  $2^4$ , and  $2^5$  on day 30 of age, respectively (data were not shown). However, the obtained antibody titres were associated with 100% protection against vNDV, under a controlled examination. As the incidence of ND in chicken flocks usually occurred at 4–5 weeks of age (Hassanzadeh & Bozorgmeri Fard, 2004), the higher proportion of chickens with protective titres would decrease the morbidity and mortality rate in the early ND challenge.

It is worth noting that the average MDA titre was higher in village chickens compared to broilers. Probably, lower biosecurity mea-

asures in native flocks than the strict conditions observed in broiler breeders cause more exposure with vNDV, resulting in a higher MDA titre in their offspring. In addition, the more rapid decreasing trend in MDA titres of broilers than that of native chickens is explained by the higher metabolic rate associated with genetic selection for more growth rate and weight gain. In accordance, Gharaibeh and Mahmoud (2013) reported that variations in the MDA half-life may stem from factors related to differences in chicken lines and types.

## 5 | CONCLUSION

In this study, a received I-2 originated ND virus successfully complied with the required specifications of a vaccinal seed. Also, administration of the produced ND.TR.IR vaccine properly raised HI antibody titres associated with a high protection level against vNDV in SPF, native, and broiler chickens. These findings suggest that not only ND.TR.IR might be useful to prevent ND outbreaks in rural flocks but also it could be advised to be administered in commercial broilers farms. However, further studies are warranted.

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## CONFLICT OF INTEREST

No potential conflict of interest is reported by the authors.

## ETHICS STATEMENT

The experiment was conducted under approval given from Razi Vaccine and Serum Research Institute (RVSRI) Ethics Committee, Karaj, Iran.

## AUTHOR CONTRIBUTIONS

*Conceptualization, methodology, supervision, and project administration:* Mohammad Abdoshah and Mohammad Hassanzadeh. *Visualization, investigation, writing—original draft, and writing—review and editing:* Shahin Masoudi and Abbas Ashtari. *Formal analysis, visualization, writing—original draft, and writing—review and editing:* Ali Reza Yousefi. *Methodology and writing—original draft:* Minoov Partovi Nasr.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.794>.

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