# Efficacy of thermostable I-2 Newcastle disease vaccine compared to B1 commercial vaccine in broiler chicken

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## Summary

Frequent vaccination failures have occurred in the broiler farms in Eurasian countries during Newcastle disease outbreaks. The disease is enzootic in many countries of the region, especially in southwest Asia. I-2 vaccine has been used successfully in village chickens in many Asian and African countries. Our preliminary study showed good efficacy of the vaccine in broiler chickens. Therefore the current experimental study was conducted to compare viral shedding period of heat resistance I-2 vaccine with B1 commercial vaccine following challenge with Herts'33. For this purpose three hundred commercial broilers were randomly allocated into four groups; 1) Thermostable I-2 vaccine, 2) Hitchner B1 vaccine, 3) Challenge group with no vaccine, and 4) Negative control group. Experimental chicks were vaccinated on days 19 and 26 by the eye drop route and then the birds were challenged via intra ocular route on day 40 with a suspension containing $10^6$ $EID_{50}$/ml challenge virus. Experimental chickens were monitored by collecting buccal and cloacal swabs at different times. Collected swabs were submitted to PCR test. The results showed that vaccination can protect the birds against mortality and also decrease virus shedding; also there was not a significant difference between vaccination with I-2 and B1 vaccines.

**Key words:** Broiler chicken, Hitchner B1 vaccine, Newcastle disease, Thermostable I-2 vaccine, Virus shedding

## Introduction

Newcastle disease is a highly contagious and fatal viral disease affecting most species of birds, especially chickens which are the most susceptible birds. The disease is frequently responsible for devastating losses in poultry (Alexander, 2000; Alexander *et al*., 2004). The etiological agent of the disease is Newcastle disease virus (NDV), also known as avian paramyxovirus type 1, which is a member of the genus Avulavirus of the Paramyxoviridae family (Mayo, 2002; Alexander and Senne, 2008).

Vaccination of poultry is known to provide an excellent means to decrease clinical disease caused by virulent NDV. Commercial available vaccines are heat sensitive and can easily be destroyed if the cold chain is not provided or is insufficient. Considering the fact that most of the Iranian climate is located in hot regions (Habibi *et al*., 2015), using heat resistant vaccines could reduce the risk of vaccination failure. The strain I-2 of NDV is a thermostable vaccine which is becoming popular due to its many advantages over other vaccines such as thermostability, easy administration by various routes such as drinking, eye drop, and mix with food, providing good protection against virulent virus (Miller *et al*., 2009; Wambura, 2009), efficient ability to transmit to non-vaccinated sensitive birds (Habibi *et al*., 2015) and good results in reducing serum concentration of acute phase proteins (Firouzi *et al*., 2014).

The objective of this research was to evaluate the effectiveness of thermostable I-2 in comparison with Hitchner B1 vaccine for protection against ND infection and virus shedding period of virulent strain of NDV, Herts'33.

## Materials and Methods

### Experimental trial

Three hundred unsexed day-old commercial broiler chicks Cobb-500® were purchased. The birds were randomly allocated into four different groups (n=75); 1) Thermostable I-2 vaccine, 2) Hitchner B1 vaccine, 3) Challenge group, and 4) Negative control group. The groups were raised in separate and isolated rooms with an optimum rearing condition in the animal lab of School of Veterinary Medicine, Shiraz University. Birds were reared on cage system, diet formulation and environmental temperature were according to cobb-500 catalog, water soluble vitamins and electrolytes were administered on first day of rearing period. Immuno-mudulator, coccidiostats orantimicrobials drugs were not used in this experiment. Similar rearing conditions were applied to all experimental groups. Birds in experimental and control groups were subjected to observation for any clinical signs throughout the experiment. The chicks were kept until 63 days old for evaluation of viral

**Table 1:** Experimental design for vaccination and challenge of different groups is presented in this table

| Group | Vaccine | First vaccination | Booster vaccination | Challenge day |
|---|---|---|---|---|
| 1 | I-2 | 19-day-old | 26-day-old | 40-day-old |
| 2 | B1 | 19-day-old | 26-day-old | 40-day-old |
| 3 | - | - | - | 40-day-old |
| 4 | - | - | - | - |

shedding and antibody response. Groups 1 and 2 received I-2 and B1 vaccines, respectively, two times with one week interval, and the groups 3 and 4 did not receive any vaccine (Table 1).

## Vaccines

Termostable I-2 vaccine was prepared in Avian Diseases Research Center, School of Veterinary Medicine, Shiraz University, and Hitchner B1 vaccine was a commercial product of Razi Vaccine and Serum Research Institute, Karaj-Iran/City/Country. I-2 is a class II genotype I virus of Australian origin used as a thermostable live vaccine, while Hitchner B1 is class II genotype II virus used as live vaccine. Experimental chicks were vaccinated by the eye drop route on days 19 and 26.

## Challenge virus

The virulent velogenic Herts'33 NDV was propagated in 9-day-old to 11-day-old embryonated chicken eggs. Concentration of the virus in infected chick embryo allantoic fluid was $10^8$ $EID_{50}$/ml. The harvested virus was diluted and birds were challenged on day 40 via intra ocular route with a suspension containing $10^6$ $EID_{50}$/ml (Villegas and Purchase, 1998).

## Clinical observation and necropsy findings

All bird groups were monitored twice daily for observing any clinical signs or death during the experiment by three persons. Necropsy was carried out on dead birds for showing the gross lesions.

## Heamagglutination inhibition (HI) test

The maternal antibody was checked by collecting serum from 4-day-old chicks. After first vaccination serum collection was done weekly to evaluate antibody response. The obtained serums from each bird were stored at -70°C until use. At each follow up time point ten birds from each group were sampled. The antibody titer in serum was determined by using 4 HA units with two-fold serum dilutions, as recommended by the World Organization for Animal Health (OIE, 2012). The I-2 vaccinal strain was used as antigen, propagated in the allantoic cavity of 9-day-old embryonated eggs.

## Virus detection

Chickens in experimental group were monitored by collecting buccal and cloacal swabs at time point of 0, 3, 6, 9, 12, 15, 18 and 21 days post challenge (dpc) and subjected to RT-PCR test. Collected swabs were placed in tubes containing 2 ml sterile normal saline (0.89% NaCl) of 7.2 pH solution. The samples were clarified by centrifugation at 4000 × g for 20 min and the supernatant was filtered by using 0.45 µm filters and were then stored at -70°C until use. Viral RNA was extracted from the filtered samples using the QIAamp® Viral RNA Mini Kit, which the extraction procedure was according to QIAGEN® protocol. Fermentas kit (Thermo Scientific RevertAidRevers Transcriptase) was used for the synthesis of first strand cDNA. RT-PCR reaction was done by AMPLIQON® Taq DNA Polymerase 2x Master Mix RED.

## Primers and reverse transcription-polymerase chain reaction

The sense primer (5´-TTG ATG GCA GGC CTC TTG C-3´) and antisense primer (5´-AGC GTC TCT GTC TCC T-3´) were chosen for the amplification of 256 bp corresponding the cleavage activation site of F gene of virulent NDV (Kant *et al*., 1997). The AMPLIQON® Taq DNA Polymerase 2x Master Mix RED was used for PCR reaction, the final volume of which was 50 µm with 40 cycles in thermal cycler.

## Statistical analysis

Heamagglutination inhibition results were analyzed by SPSS 21 using ANOVA.

# Results

## Clinical signs and survival rate

All birds were clinically healthy before challenge. Protection from challenge virus was determined by the absence of clinical signs and death during the 21 dpc period. Birds in the unchallenged control group (group 4) had no clinical signs during the course of the experiment. The birds appeared clinically normal until 3 dpc in the groups 1, 2 and 3. Most birds in challenged groups (1, 2 and 3) showed clinical signs 4 to 8 days after inoculation. All birds in the unvaccinated-challenged group (group 3) displayed conjunctivitis, severe depression, respiratory signs, greenish diarrhea, ruffled feathers and decrease in feed intake from day 3 pc. Some birds died shortly after the appearance of clinical signs, or even died overnight without any noticeable signs. Chickens in vaccinated-challenged groups showed much less clinical signs compared to unvaccinated-challenged group, however vaccinated groups showed some degree of conjunctivitis, mild nasal discharge and depression. Leg paralysis was observed in 3% (the number of 2/75) of chickens in groups 2 from day 5 pc. No mortality was observed in negative control group while all birds in unvaccinated-challenged group died during the course of the experiment. Both I-2 and B1 commercial vaccines were

effective in preventing mortalities. However birds in unvaccinated-challenged group showed 100% mortality (Fig. 1).

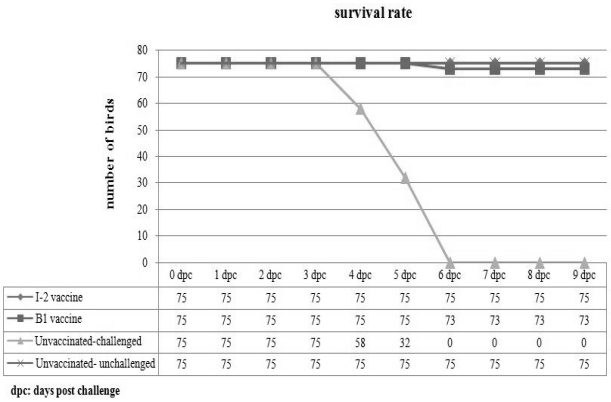


**Fig. 1:** Survival rate in broiler chickens vaccinated with I-2 and B1 Newcastle disease vaccines compared to unvaccinated-challenged birds. As indicated in the figure unvaccinated-challenged birds showed 100% mortality

## Necropsy findings

At necropsy, all dead birds, from unvaccinated-challenged (group 3), showed typical lesions similar to natural infected field cases from day 4 pc. Gross pathological lesions mainly in gastrointestinal tract were observed. There was oedema in mucosal surface of trachea, petechial and necrotic haemorrhages of the proventriculus, intestine, caecum and caecal tonsils, and deep-green content of the gastrointestinal tract starting from the proventriculus that ended up with green faeces. In addition petechial hemorrhage on the serosa surface of the heart could be seen in some cases in this group. The lesions of dead birds in group 2 had less severity in comparison with group 3. The dead birds in group 2 (B1 vaccine group) were hyperemic and there were mild congestion of the trachea and some petechial hemorrhages on cecal tonsils and the proventicular glands.

## Serological findings

The chicks had mean maternal antibody titer of 8 on 4-day-old chicks in HI examination. The mean of serum antibody titers ($Log_2$±SE) against NDV antigen in all experiment groups is presented in Table 2.

## Virus shedding

The data of challenged group virus shedding are shown in Fig. 2 and Table 3. In all challenged groups, the numbers of positive cloacal swaps were more than buccal ones. Virus shedding in unvaccinated-challenged group was significantly (should be emitted) more than vaccinated-challenged groups.

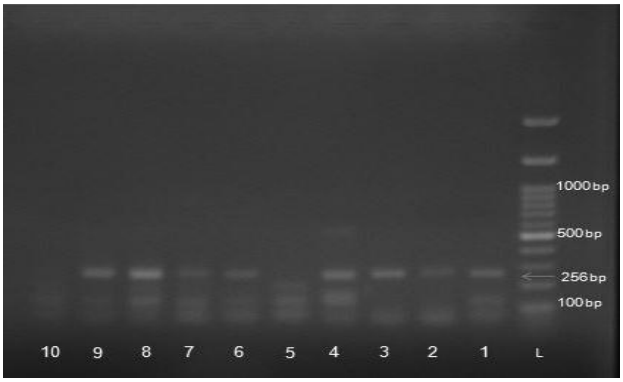


**Fig. 2:** Negative (Lanes 5 and 10) and positive samples (Lanes 1, 2, 3, 4, 6, 7, 8, 9) of buccal and cloacal swabs taken 6 (Lanes 1, 2, 3, 4, 9) and 9 (Lanes 5, 6, 7, 8, 10) days post challenge are shown in this figure. Buccal swabs: Lanes 2, 4, 6, 8; cloacal swabs: Lanes 1, 3, 5, 7, 9, 10; I-2 groups (Lanes: 1, 2, 5, 6); B1 groups (Lanes 3, 4, 7, 8). Lane 9 belongs to unvaccinated-challenged, and Lane 10 belongs to negative control group. Lane L: Ladder 100 bp

**Table 2:** Mean HI titer ($Log_2$±SE) of serum in all experimental groups before and after vaccination/challenge on selected days of age

| No. | Group | After vaccination | | | | | | After challenge | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 4* | First vaccine | | Booster vaccine | | | 3 dpc | 7 dpc | 14 dpc |
| | | | 3 dpv | 7 dpv | 3 dpv | 7 dpv | 14 dpv | | | |
| | | | 22 | 26 | 29 | 33 | 40 | 43 | 47 | 54 |
| 1 | I-2 vaccine | 8±0.55$^{a}$ | 1.8±0.23$^{a}$ | 3.6±0.20$^{a}$ | 4.9±0.19$^{a}$ | 5.5±0.09$^{a}$ | 6.1±0.08$^{a}$ | 7.4±0.21$^{a}$ | 7.5±0.18$^{a}$ | 7.5±0.10$^{a}$ |
| 2 | B1 vaccine | 8±0.55$^{a}$ | 1.7±0.30$^{a}$ | 3.5±0.21$^{a}$ | 4.7±0.19$^{a}$ | 5.4±0.10$^{a}$ | 5.9±0.08$^{a}$ | 7.3±0.29$^{a}$ | 7.6±0.19$^{a}$ | 7.6±0.10$^{a}$ |
| 3 | Unvaccinated-challenged | 8±0.55$^{a}$ | 0.7±0.30$^{b}$ | 0.7±0.30$^{b}$ | 0.5±0.10$^{b}$ | 0.5±0.10$^{b}$ | 0.5±0.10$^{b}$ | 3.8±0.92$^{b}$ | - | - |
| 4 | Unvaccinated-unchallenged | 8±0.55$^{a}$ | 0.7±0.30$^{b}$ | 0.7±0.30$^{b}$ | 0.5±0.10$^{b}$ | 0.5±0.10$^{b}$ | 0.5±0.10$^{b}$ | 0.5±0.10$^{c}$ | 0.2±0.08$^{b}$ | 0.2±0.08$^{b}$ |

* Days of age. dpv: Days post vaccination, dpc: Days post challenge, $^{abc}$ Different superscripts shows statistical significance (P≤0.05) between groups in each column

**Table 3:** Virus shedding and survival rate of different groups after challenge with virulent NDV (Herts'33)

| | 0 dpc | | | 3 dpc | | | 6 dpc | | | 9 dpc | | | 12 dpc | | | 15 dpc | | | 18 dpc | | | 21 dpc | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shed | | Dead | Shed | | Dead | Shed | | Dead | Shed | | Dead | Shed | | Dead | Shed | | Dead | Shed | | Dead | Shed | | Dead |
| | B | C | | B | C | | B | C | | B | C | | B | C | | B | C | | B | C | | B | C | |
| I-2 vaccine (I) | 0/5 | 0/5 | 0/75 | 1/5 | 3/5 | 0/75 | 5/5 | 5/5 | 0/75 | 3/5 | 2/5 | 0/75 | 1/5 | 1/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 |
| B1 vaccine (II) | 0/5 | 0/5 | 0/75 | 2/5 | 4/5 | 0/75 | 5/5 | 5/5 | 2/75 | 3/5 | 3/5 | 0/73 | 1/5 | 3/5 | 0/73 | 0/5 | 1/5 | 0/73 | 0/5 | 0/5 | 0/73 | 0/5 | 0/5 | 0/73 |
| Unvaccinated-challenged (III) | 0/5 | 0/5 | 0/75 | 5/5 | 5/5 | 0/75 | 5/5 | 5/5 | 75/75 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Unvaccinated-unchallenged (IV) | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 | 0/5 | 0/5 | 0/75 |

Shed: Number of birds shedding virus/total samples taken, Dead: Number of dead birds/the rest of alive birds, dpv: Days post vaccination, dpc: Days post challenge, B: Buccal swab, and C: Cloacal swab

## Discussion

Although intensive vaccination programs and different strains of vaccines are applied, the ND is enzootic in commercial poultry of Iran and it has continued for years. ND continues to be a major threat for the poultry industry and annual losses due to the disease and cost related preventive strategies indicate the need for continuous research on vaccine type and efficacy of vaccines against circulating NDV. In this study, it was assessed whether thermostable I-2 vaccine can induce the humoral immunity in chicks compared to Hitchner B1 against Herts'33, a virulent strain.

Both I-2 and B1 vaccines were generally safe with no adverse effect in the vaccinated birds. However, our personal field experience on application of I-2 vaccine in commercial broiler birds subjected to respiratory complex infections was associated with much less advert clinical manifestation, compared to other commercial vaccines available to poultry farmers. Also, it was seen that both vaccines provided chicks with full protection from overt clinical disease caused by the challenge virulent NDV. No mortality was observed in any of the chicks that had received either of the vaccines. The findings were consistent with the previous reports (Nasser *et al*., 2000; Degefa *et al*., 2004; Kapczynski and King, 2005; Van Boven *et al*., 2008; Fentie *et al*., 2014), which demonstrated that vaccination produced full protection from disease caused by virulent NDV in their experimental works.

No clinical signs or virus excretion was observed in any birds of the unchallenged control group. The unvaccinated-challenged birds demonstrated clinical signs and gross lesions of the disease as well as virus excretion from 3 dpc and finally had total mortality (100% mortality). This confirms that vNDV strains are capable of causing high mortality in unvaccinated susceptible flocks which is consistent with the idea suggested by others (Young *et al*., 2002; Alexander *et al*., 2004).

Both vaccinated groups had lower mortality rate than unvaccinated-challenged group, in which full protection from death was only observed in birds of group 1 (no mortality) which received I-2 vaccine strain followed by group 2 with 3% mortality; however, in comparison of these two vaccine strains it was not statistically significant.

Comparison on the beginning and severity of the clinical signs of the disease among different groups demonstrates that two vaccinated-challenged groups showed clinical signs later (from day 5 pc) and less severe than the unvaccinated-challenged group. In addition, necropsy findings revealed that lesions were less severe in the vaccinated group 2 as compared with positive control group.

While some birds in vaccinated-challenged groups were protected from clinical disease and mortality, they were not protected against infection since they shed challenged virus in faecal and buccal samples. Our findings were consistent with the reports of others (Kapczynski and King, 2005; Van Boven *et al*., 2008; Fentie *et al*., 2014). The chicks in two vaccinated-challenged groups excreted challenge virus from day 3 dpc and suggested that vaccinated chicks may still act as reservoirs and be a source of NDV infection for susceptible birds. However, the incidence and duration of virulent virus shedding in infected birds of groups were different. Vaccination decreased the rate of virus excretion. Similar findings were reported by other researches (Kapczynski and King, 2005; Fentie *et al*., 2014). High antibody level produced in vaccinated-challenged groups may be responsible for reduced virulent virus shedding.

There was not any statistically significant difference between two strains of vaccines regarding shedding periods, however the shedding days compared to B1 group were lower in I-2 vaccine group and can be a good candidate in field condition of Iran.

Although there has not been any significant difference (in mortality, clinical signs and virus shedding) between vaccination with I-2 and B1 vaccines, considering the fact that frequent vaccination failures occur in field condition due to sensitivity of commercially available vaccines because of inability to provide cold chain, I-2 which is heat resistant can be a proper candidate to campaign against ND in tropical areas of Iran.

This study showed that vaccination with thermostable I-2 can be a proper candidate to campaign against ND in tropical areas of Iran.

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