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Effectiveness of different Newcastle disease vaccination programs in Iranian broiler farms: a case-control study

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A B S T R A C T

Due to its high prevalence and rapid spread, Newcastle disease is considered a deadly viral disease for the poultry industry worldwide. The uncontrolled and unplanned use of the Newcastle vaccine by poultry farmers reduces its protective value. This study aims to calculate the effectiveness of vaccination programs against Newcastle disease in Iranian broiler farms. The study method is analytical and case-control. One hundred two case farms and 102 control farms were selected for the study. The case farm was defined as a farm with losses exceeding 7%, accompanied by clinical criteria, post-mortem examination, molecular testing, and a history of regional disease outbreaks. The control farms had natural losses (below 7%) and no history of any disease in the current and previous periods. Control farms were matched with case farms regarding capacity, breed, and provincial distribution. The data were analyzed using the chi-square test and multivariable logistic regression analysis. The statistical software Stata version 14 was used for data analysis and modeling. According to the results, the use of the Vitapest strain vaccine by the spray method on day one, followed by the use of the inactivated vaccine by the injection method, and then two doses of the LaSota strain vaccine by the oral route in the program, (87% with a 95% confidence interval of 35-98%), the use of the inactivated vaccine by the injection method and two doses of LaSota strain vaccine by the oral route, (84% with a

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95% confidence interval of 23-97%), the use of the inactivated Newcastle disease vaccine at the age of 7–12 days, (78% with a 95% confidence interval of 25-94%), and the use of the live vaccine against Newcastle disease with more than two doses in the program (66% with a 95% confidence interval of 15-88%) were effective. According to the results of this study, inactivated Newcastle disease vaccines are ineffective in the first week, and it is better to administer them at the ages of 7 to 12 days. It is also recommended to use spray vaccines of viscerotropic strains at one day of age to increase mucosal immunity against the Newcastle virus. Finally, principles of biosecurity and farm hygiene management should also be considered to achieve maximum vaccine effectiveness and have a consistent and regular program on the farm.

Keywords: *Effectiveness, Newcastle disease, broiler chickens, case-control study, vaccine*

1 Introduction

The causative agent of Newcastle disease is Avian Paramyxovirus-1 (PMV-1). This virus belongs to the *Paramyxoviridae* family, under the *Paramyxovirinae* subfamily and *Avulavirus* genus (1, 2). Prevention and control of Newcastle disease are based on two principles: health and management measures in the flock, compliance with biosecurity, and proper vaccination program management and design. In addition to following biosecurity principles and eliminating infected birds, vaccination is an integral part of Newcastle disease control programs (3).

Despite easy access and global implementation of vaccination against Newcastle disease since the 1950s, the disease remains a threat to poultry producers and many other bird species worldwide (4). Vaccination is carried out individually and collectively in poultry. Mass vaccination methods such as drinking water, spray, and aerosol are more cost-effective and less labor-intensive than injecting vaccines into each bird. Unfortunately, achieving the desired antibody titer in a high percentage of birds in the flock is challenging with live group vaccination (5).

Immunity against Newcastle disease is achieved through circulating antibodies in the blood, secretory antibodies that provide mucosal immunity, and cell-mediated immunity. Live Newcastle disease vaccines contain live viruses that can infect cells and mimic natural infection in the bird's body, stimulating all three types of immune responses. The ability of viruses to infect cells is eliminated in killed vaccines by chemical agents, radiation, and heat. These vaccines only stimulate humoral immune responses (6).

The methods for evaluating the effectiveness of a vaccination program vary widely and generally involve assessing and monitoring all aspects of health. The presence of disease outbreaks and mortality is often used as a criterion for success. Vaccination programs' ineffectiveness for clinically infected or moribund birds is quite apparent in some endemic regions with highly pathogenic organisms (such as Newcastle disease virus with visceral tropism). However, in most regions of the world, the low desirability of vaccination programs is less observed. In this regard, minimizing the risk of disease involvement and maximizing production efficiency economically and practically with an effective program is necessary. Many aggressive vaccination programs that provide high levels of protection are costly and detrimental to the growth rate. The goal of veterinarians and other health professionals is to regulate these criteria as much as possible and effectively (5).

Newcastle disease is endemic in Iran, and the most critical control measure is the widespread use of various common vaccines. This study aims to compare the most common vaccination programs used against Newcastle disease in poultry farms in the country to calculate their effectiveness and determine the best method, recommending it to poultry producers. Additionally, it discusses the impact of compliance with management principles and biosecurity on the effectiveness of implemented programs.

2 Materials and Methods

2.1 Study design and sample size

This study was designed as an analytical and case-control study conducted throughout the country for two years, from September 2015 to September 2017. The target population or the study population was poultry farms across the country. The number of farms studied was determined using the sample size calculation formula for case-control studies, taking into account the exposure ratio (to inactivated

vaccine) in the control group as 62% and the exposure ratio in the case group as 42%, with a significance level of 5% and a study power of 80%. The required number of units for this study was calculated to be 102 case farms and 102 control farms.

2.2 Selection of the case group:

Based on the veterinary report of the farm, a broiler farm with a mortality rate of more than seven percent and suspicion of Newcastle disease that met the following criteria was selected as the case group:

1. History of Newcastle disease outbreak in the area.
2. Clinical criteria: Clinical signs and specific post-mortem findings of Newcastle disease, including respiratory form: gasping, coughing, respiratory sounds, nasal hemorrhage, inflamed and hemorrhagic air sacs, or gastrointestinal form: decreased water and feed intake, green diarrhea, ulceration and hemorrhage in the proventriculus and cecal tonsils, plaque in the intestine, or neurological form: head and neck twisting, muscular tremors, sitting on the ground, paralysis of wings and legs, or a combination of these observed symptoms.
3. Laboratory criteria: An RT-PCR test was performed to determine the presence of Newcastle disease virus (wild type) on swab samples taken from affected birds on the farm.

After reporting the disease, necessary sampling was performed by visiting the selected farm, and a designed questionnaire was also completed. In each unit, tissue samples, including trachea, lung, spleen, cecal tonsils, and head, were obtained from 5-7 freshly dead birds. The collected samples were sent to the reference laboratory of the Avian Disease Diagnosis Center for testing. Farms confirmed positive for Newcastle disease after molecular testing and met the above criteria were considered cases.

2.3 Selection of the control group:

To select control farms, farms from the same region as the identified farm were chosen. Broiler farms active during the previous study period had regular mortality rates (below 7 percent). They did not show clinical signs of Newcastle disease or other similar diseases associated with Newcastle disease (respiratory complex) were selected as controls. These farms were monitored until the end of the rearing period, and if they did not face Newcastle or a similar disease by the end of the period, they were selected as controls.

The case and control groups were matched based on poultry breed and farm capacity. The study farms were located in twenty provinces of the country: West Azerbaijan, Alborz, Isfahan, Ilam, North Khorasan, Razavi Khorasan, South Khorasan, Khuzestan, Zanjan, Semnan, Fars, Qazvin, Kurdistan, Kerman, Gilan, Golestan, Lorestan, Mazandaran, Hormozgan, and Yazd. The selection of the study farms was targeted and based on the reports of new disease cases that were received.

2.4 Data collection

The required data for the research study were collected using a questionnaire based on expert opinions and a literature review. The questionnaire consisted of six pages and included both closed and open-ended questions. The questionnaire included 5 parts: General information about the poultry farm unit, Technical information about the unit, Rearing period information, Disease information, Clinical, post-mortem, and laboratory findings. The variables in the questionnaire included flock characteristics (age, flock size, ventilation system, and heat and humidity sources), flock hygiene (disinfection of water and rearing facility, presence of other diseases in the flock, fencing, and the presence and type of carcass disposal system), and information related to vaccination methods, number of vaccinations, vaccine type, and manufacturer. The questionnaire was completed after visiting the poultry farm and observing and interviewing the owner or farm veterinarian. The laboratory results were recorded on the relevant forms after the tests were conducted. The reliability of the questionnaire was assessed using Cronbach's alpha test on 30 questionnaires, and a reliability coefficient of 0.7 was obtained (α=0.7).

2.5 Statistical Analysis

For data analysis, quantitative variables were categorized into two groups using the median as the cut-off for qualitative variables. All independent variables were analyzed using chi-square tests or Fisher's exact test. Multivariable analysis was performed using a logistic regression model. Variables with a p-value less than 0.2 in the univariable analysis were selected for entry into the multivariable analysis. The correlation between candidate variables for multivariable analysis was examined using the Collinearity test and calculating the variance inflation factor (VIF). A VIF greater than ten was considered a severe correlation (7), and one of the variables was removed from the multivariable analysis. The variable that had a stronger

association with the desired outcome was included in the model. Multivariable analysis was performed using the backward LR method, and $p<0.05$ was considered statistically significant. The final model fit was tested using the Hosmer and Lemeshow Test, and the model with the highest p-value was selected as the final model. All statistical analyses were conducted using Stata software version 14 (Stata Corp, College Station, TX). The vaccine effectiveness was calculated using the VE= 1- OR *100 formula.

3 Results

In this study, a total of 204 farms, including 102 case farms and 102 control farms, were sampled. The mean ± standard deviation of farm capacity in the case group was 21554.9 ± 13231.4 pieces, and in the control group was 22109.8 ± 13011.6. The highest frequency of poultry breeds under study in the case and control groups was Ross (66%) and Ross 308 (20%), respectively, and the lowest frequency was related to the Cobb (14%).

The frequency distribution, regression coefficient, correlation power (odds ratio), and significance level of the independent variables under study in the single-variable regression model are presented in Table 1. Based on the results of the univariable analysis, variables with $p<0.05$ included the age of using an inactivated vaccine, type of vaccine strain and administration method in three vaccination sessions, use of digestive and then respiratory strains in the farm, interval between using two live vaccines of Newcastle disease and infectious bronchitis for five days or more, median number of Newcastle disease vaccinations before 10 days of age, place of production of inactivated Newcastle disease vaccines, median age of starting Newcastle disease vaccination, median number of live Newcastle disease vaccinations in the farm, and compliance with health management and biosecurity principles, which were subjected to multivariable analysis.

Table 1. Univariable analysis of some of the effective factors on different Newcastle disease vaccination programs effectiveness in Iranian broiler farms

variable	Category	Case (%) N=102	Control (%) N=102	[b]OR (95% CI)	[c]P-value
Age of killed vaccine usage (day)	1	9(8.8)	13(12.7)	0.28(0.09-0.89)	0.03
	2-6[a]	22(21.6)	9(8.8)	1	-
	7-12	40(39.2)	52(51.0)	0.31(0.13-0.75)	0.01
	Not using a killed vaccine	31(30.4)	28(27.5)	0.45(0.17-1.14)	0.09
Type of vaccine strains and route of administration of Newcastle disease vaccine	Injection killed vaccine+ eye drop b1[a] Drinking LaSota Drinking LaSota	21(20.6)	14(13.7)	1	-
	Injection killed vaccine Drinking LaSota Drinking LaSota	5(4.9)	12(11.8)	0.27(0.08-0.96)	0.04
	Injection killed vaccine Eye drop b1 Drinking b1	7(6.9)	2(2.0)	2.33(0.42-12.91)	0.33
	PHY.LMV.42 spray Injection killed vaccine+ eye drop b1 Drinking LaSota	10(9.8)	20(19.6)	0.33(0.12-0.92)	0.03
	PHY.LMV.42 spray Drinking LaSota Drinking LaSota	3(2.9)	12(11.8)	0.16(0.04-0.70)	0.01
	Drinking b1 Drinking Clone Drinking LaSota	8(7.8)	6(5.9)	0.88(0.25-3.12)	0.85
	PHY.LMV.42 spray Injection killed vaccine Drinking LaSota Drinking LaSota	5(4.9)	11(10.8)	0.3(0.08-1.06)	0.06

	Other programs	43(42.2)	25(24.5)	1.14(0.49-2.64)	0.74
The first vaccine with gastrointestinal tropism	No[a]	77(75.5)	62(60.8)	1	-
The second vaccine with respiratory tropism	Yes	25(24.5)	40(39.2)	0.5(0.27-0.91)	0.02
Newcastle live vaccine Use drinking rout after seven days	No[a]	14(13.7)	9(8.8)	1	-
	Yes	76(74.5)	85(83.3)	0.57(0.23-1.40)	0.22
	Not using	12(11.8)	8(7.8)	0.96(0.28-3.28)	0.95
The interval between the use of two live vaccines, Newcastle and Bronchitis, five days and more	No[a]	19(18.6)	9(8.8)	1	-
	Yes	50(49.0)	42(41.2)	0.56(0.23-1.37)	0.20
	Not using a bronchitis vaccine	33(32.4)	51(50.0)	0.30(0.12-0.75)	0.01
Use all tropisms against Newcastle disease in the program	No[a]	62(60.8)	45(44.1)	1	-
	Yes	40(39.2)	57(55.9)	0.5(0.29-0.88)	0.01
Use eye drops with pneumotropic or pneumo-viscerotropic strain	No[a]	46(45.1)	56(54.9)	1	-
	Yes	56(54.9)	46(45.1)	1.48(0.85-2.57)	0.16
Use a spray with a viscerotropic strain of one-day-old broiler	No[a]	77(75.5)	51(50.0)	1	-
	Yes	25(24.5)	51(50.0)	0.32(0.17-0.58)	0.0001
Drinking LaSota two times in the vaccination program	No[a]	74(72.5)	38(37.3)	1	-
	Yes	28(27.5)	64(62.7)	0.22(0.12-0.40)	0.0001
Manufacturer of killed Newcastle vaccine	Inside the country	22(21.6)	48(47.1)	1	-
	abroad	49(48.0)	26(25.5)	4.11(2.05-8.22)	0.0001
	Not using the killed vaccine	31(30.4)	28(27.5)	2.41(1.17-4.95)	0.01
Manufacturer of killed Newcastle vaccine	Inside the country	16(16.8)	20(23.5)	1	-
	abroad	79(83.2)	65(76.5)	1.51(0.72-3.16)	0.26
Median of vaccination age (day)	≤4[a]	43(42.2)	57(55.9)	1	-
	>4	59(57.8)	45(44.1)	1.73(0.99-11.23)	0.05
Median of the total number of times that the Newcastle vaccine was used	≤3[a]	66(64.7)	56(54.9)	1	-
	>3	36(35.3)	46(45.1)	0.66(0.37-1.16)	0.15
The median of the total number of times that live Newcastle vaccine used	≤2[a]	59(57.8)	44(43.1)	1	-
	>2	43(42.2)	58(56.9)	0.53(0.31-0.96)	0.03
Median of health management and biosecurity index	Inappropriate	79(77.5)	32(31.4)	1	-
	Appropriate	23(22.5)	70(68.6)	0.13(0.07-0.24)	0.0001

[a]Reference group. Odds ratio (confidence interval for OR). [c]P<0.05 was considered as statistically significant.

The variables of having two doses of LaSota vaccine by drinking water method in the program, using vaccine with viscerotropic strain by spray method at one day old, and using Pneumotropic or Pneumovisrotropic vaccine by ocular drop method were not included in the multivariable analysis due to their correlation with the vaccine strain and administration method in three vaccination sessions.

To create a hygiene and biosecurity management index variable, a combination of variables with p<0.05 in the univariableanalysis was used, including variables of appropriate fencing, presence of salon disinfection and bedding, presence of an incinerator for disposal of contaminated carcasses, presence of a moisture source, origin of purchasing feed outside the farm and in ready-to-use form, and presence of a longitudinal ventilation system in the salon.

Table 2 shows the results of the multivariable logistic regression analysis obtained from the univariate analysis with independent variables having a significance level of less than 0.05. The odds ratio of having appropriate hygiene and biosecurity management {OR=0.11 (95%CI: 0.04-0.25), p<0.0001} was obtained, considering the relationships between independent variables affecting the effectiveness of vaccination and independent of the vaccine, such as the hygiene and biosecurity management variable, which was considered as a confounding variable in this study.

Table 2. Multivariable Logistic regression analysis of variables associated with the effectiveness of different Newcastle disease vaccination programs in Iranian broiler farms

variable	Category	[b]OR (95% CI)	[c]P-value
Age of killed vaccine usage (day)	1	0.49(0.10-2.30)	0.36
	2-6[a]	1	-
	7-12	0.22(0.06-0.75)	0.01
Type of vaccine strains and route of administration of Newcastle disease vaccine	Injection killed vaccine+ eye drop b1[a] Drinking LaSota Drinking LaSota	1	-
	Injection killed vaccine Drinking LaSota Drinking LaSota	0.16(0.03-0.77)	0.02
	Injection killed vaccine Eye drop b1 Drinking b1	4.83(0.68-34.42)	0.11
	PHY.LMV.42 spray Injection killed vaccine+ eye drop b1 Drinking LaSota	0.58(0.15-2.25)	0.43
	PHY.LMV.42 spray Drinking LaSota Drinking LaSota	0.17(0.01-1.62)	0.12
	Drinking b1 Drinking Clone Drinking LaSota	0.83(0.10-6.50)	0.86
	PHY.LMV.42 spray Injection killed vaccine Drinking LaSota Drinking LaSota	0.13(0.02-0.65)	0.01
	Other programs	0.56(0.16-1.92)	0.35
The median of the total number of times that live Newcastle vaccine used	≤2[a]	1	-
	>2	0.53(0.31-0.96)	0.03
Median of health management and biosecurity index	Inappropriate	1	-
	Appropriate	0.13(0.07-0.24)	0.0001

[a]Reference group. Odds ratio (confidence interval for OR). [c]P<0.05 was considered as statistically significant. Hosmer-Lemeshow χ^2 (8) =4.66, P-value=0.79.

The factors associated with vaccine effectiveness and related to the vaccine were determined in the studied broiler farms. The vaccination program included the use of Vitapest strain vaccine by spray method at one day old, followed by killed injection vaccine and then two doses of LaSota strain vaccine by drinking method {OR=0.13 (95%CI: 0.02-0.65), P=0.01}. The vaccination program also included the use of killed injection vaccine and two doses of LaSota strain vaccine by drinking method {OR=0.16 (95%CI: 0.03-0.77), P=0.02}, and the use of killed vaccine at 7-12 days old {OR=0.22 (95%CI: 0.06-0.75), P=0.01}, and using the live Newcastle disease vaccine more than two times in the program {OR=0.34 (95%CI: 0.12-0.85), P=0.02} were protective factors. They reduced the risk of Newcastle disease and enhanced the effectiveness of vaccination against this disease, thus considered protective factors. None of the variables in the multivariable analysis had a significant interaction effect.

The effectiveness of variables related to the Newcastle disease vaccination program, which were found to be significant in the final model, is presented in Table 3. According to the final model, the vaccination program that included the use of Vitapest strain vaccine by spray method at one day old, followed by killed injection vaccine and then two doses of LaSota strain vaccine by drinking method, had an effectiveness of 87% (95% CI: 35-98). The vaccination program that included the killed injection vaccine and two doses of LaSota strain vaccine by drinking method had an

effectiveness of 84% (95% CI: 23-97). The vaccination program that included the killed Newcastle disease vaccine at 7-12 days old had an effectiveness of 78% (95% CI: 25-94). The vaccination program that included the use of live Newcastle disease vaccine more than two times had an effectiveness of 66% (95% CI: 15-88).

Table 3. Effectiveness of different Newcastle disease vaccination programs in Iranian broiler farms

Vaccination program	Case (%) N=102	Control (%) N=102	[a]OR (95% CI)	[b]VE (95% CI)
Injection killed vaccine Drinking LaSota Drinking LaSota PHY.LMV.42 spray Injection killed vaccine Drinking LaSota Drinking LaSota	5 (4.9)	12 (11.8)	0.16 (0.03-0.77)	84% (23-97)
Age of killed vaccine use at 7-12 days	40 (39.2)	52 (51.0)	0.22 (0.06-0.75)	78% (25-94)
Use live Newcastle vaccine more than two times in the vaccination program	43 (42.2)	58 (56.9)	0.34 (0.12-0.85)	66% (15-88)

[a]Odds ratio (95% confidence interval). [b]Vaccine effectiveness (95% confidence interval). VE= (1-OR) ×100

4 Discussion

Newcastle disease has been identified in Iran since 1941, and it causes significant economic losses to the poultry industry in Iran every year. Extensive vaccination is used in the commercial poultry sector to control the disease, particularly in broilers. However, despite this widespread vaccination, disease outbreaks are observed yearly in the country. Biosecurity measures and an effective and efficient vaccination program in poultry farms should be implemented to succeed in disease control. According to the results of the present study, the vaccination program that includes the use of Vitapest strain vaccine by spray method at one day old, followed by killed injection vaccine and then two doses of LaSota strain vaccine by drinking method, the vaccination program that includes the use of killed injection vaccine and two doses of LaSota strain vaccine by drinking method, the vaccination program that includes the use of killed Newcastle disease vaccine at 7-12 days old, and the vaccination program that includes the use of live Newcastle disease vaccine more than two times in the program have shown suitable effectiveness against Newcastle disease.

Based on this study's results, injectable vaccines in chicks older than 7 days have been effective. The study conducted on the age of killed vaccine use in this disease in broiler chicks indicates that vaccinating chicks at one day old in the presence of maternal antibodies creates weak immunity against the virus, and using the vaccine at the age of ten days with a lower level of maternal antibodies in the serum allows for better protection against the virus. In this study, it was recommended to use the killed influenza vaccine at 7-10 days old, or if used at one day old, administer two doses of the killed vaccine (8). Several reasons contribute to this effective finding. Maternal immunity impacts live and killed vaccines (9, 10). Therefore, it is better not to use killed vaccines early, like live vaccines. In addition to the impact of maternal immunity, it seems that injecting killed vaccine at an early age of chicks, due to increased vaccination errors, small chick size, thymus damage in the neck, delay in closing the injection site, and vaccine leakage may not be effective and efficient. In addition to the mentioned reasons, the inability of B cells, which produce circulating antibodies, to respond at an early age can also support this issue. The study conducted on the evolution of the avian immune system in creating effective antibodies against Newcastle disease concluded that the immature immune system of young chicks up to seven days old will not produce an effective antibody response against this disease. In this study, one-day-old broiler chicks were exposed to Newcastle disease antigen on days one, seven, and 12. The serum level of antibodies produced in the serum was measured from the seventh day after exposure until day 28. Significantly lower levels of antibodies were produced in the chicks exposed at one day old compared to the other two groups. The results of this study indicate the inability of B cells, which results in antibody production in chicks vaccinated at one day old (11).

A study was conducted in Pakistan (2014) to assess the efficacy of killed vaccines versus live vaccines against Newcastle disease and the age of vaccine administration. The results of this study showed that the efficacy of the

killed Newcastle disease vaccine against the virulent viscerotropic strain was 90% to 100%. In comparison, the efficacy of the live vaccine was 60%, indicating a significant difference between the two. In this study, vaccination with the killed vaccine was administered orally to the first group at five days of age, followed by a Newcastle-bronchitis bivalent vaccine at seven days of age, and a reminder of the killed Newcastle disease vaccine was injected at 18 days of age. In the second group, a Newcastle-bronchitis bivalent live vaccine was administered orally at seven days, followed by two cloned Newcastle vaccines at 18 and 28 days of age. The results of this study regarding the age of killed vaccine administration contradict the present study's findings. However, according to this study's results, the presence of the killed vaccine in the program enhances its effectiveness (12).

In a study by Sarcheshmei et al. in 2016, the highest efficacy of vaccination programs was reported for administering the killed vaccine at eight days of age, consistent with the results of the present study (13). In the country, the Newcastle disease vaccine is used in poultry as a killed vaccine, along with the influenza vaccine in the form of a bivalent vaccine. In a study on avian influenza (2010), the use of the killed vaccine and two doses of the live LaSota strain vaccine administered orally against Newcastle disease was found to increase its effectiveness. This result is consistent with the studies by Jang et al. (1999) and Ahmed et al. (2007) (11, 14). The live vaccine, followed by the kill vaccine, provides a higher and more stable titer against the Newcastle disease virus (15).

In 2004, Dukfa et al. conducted a study on Newcastle vaccines' efficacy and economic and technical efficiency. They concluded that vaccination using the eye drop method on the first day of the chick's life, followed by a similar dosage at three weeks old, would significantly produce high antibody levels. The highest antibody titer (log2, 6.6) was achieved, and 93% of the chicks were protected against the challenge of the virulent field virus. The spray method resulted in a lower antibody titer (log2, 5.9), with only 53% of the chicks remaining protected against the challenge of the live virus. These results indicate that the eye drop method is preferred over the drinking water and spray methods (16), which contradicts the present study's findings. There was no significant relationship between using the pneumotrop strain vaccine via the eye drop method and reducing Newcastle disease incidence (OR= 1.48, P= 0.16).

In the present study, using the viscerotropic vaccine via the spray method at one day old significantly reduces the chance of Newcastle disease infection (OR= 0.32, P= 0.001). Using the viscerotropic vaccine via the spray method, followed by injection with the killed vaccine, and then two doses of the LaSota strain vaccine via the drinking water method, provides up to 87% protection against Newcastle disease virus during the rearing period. These findings are consistent with a study conducted by Prozo et al., in 2008. In that study, they used the pneumovisurotropic Avinew vaccine strain to assess mucosal immunity and protection against Newcastle disease virus challenge. They found that early vaccination with the Avinew vaccine, regardless of maternal antibodies, provides 95% to 100% protection against the virus (17).

The importance of mucosal immunity in the defense mechanisms against viral diseases has been emphasized recently (18). Mucosal immunity acts as a barrier in the early stages of infection, preventing viral spread (19). Among vaccination methods, mucosal vaccination has received more attention because, in addition to systemic immunity, it induces local immunity involving IgM, IgA, and IgG, similar to mucosal cellular immune responses. The locally generated immunity protects against Newcastle disease virus infection in the respiratory mucosal tissues, the primary target tissues for this virus (20). This finding is attributed to maternal immunoglobulin G in tears, which is relatively low in mucosal levels, allowing for the administration of the spray method from day one. The spray vaccine method compensates for maternal immunity weakness and affects local immunity. Local immunity, expressed by IgA, plays an influential role in combating the Newcastle disease virus (21).

Swayne (2013), in the book "Diseases of Poultry," considered the proposed vaccination program for broiler chickens as a single dose of live vaccine at one day old, along with one or two booster doses during the rearing period. These recommendations align with the current study's findings regarding the number of times live vaccines are used exceeding two doses in the program (9).

5 Conclusion

This study has increased our knowledge regarding the level of immune response protection against Newcastle disease in different vaccination programs and provided valuable information about developing and evaluating different vaccination methods. Based on the results of this study, the following recommendations can be made to poultry farmers, veterinarians, and individuals working in

this industry: Killed vaccines against Newcastle disease should not be used in the early stages of chick's life but should be administered at 7 to 12 days of age, with an average of 10 days. At one day old, the viscerotropistrain vaccine should be administered using spray to enhance mucosal immunity against the Newcastle virus. Using two doses of live LaSota strain vaccine through drinking water from seven days onwards significantly reduces this disease and its associated damages in the country's poultry industry. Considering the situation of Newcastle disease in the country, it is recommended to use live vaccines against this disease more than two times during the rearing period, with appropriate intervals, alongside killed vaccines. Finally, principles of biosecurity and farm health management should also be considered to achieve maximum vaccine effectiveness and have a consistent and regular program on the farm. Adhering to principles of biosecurity and farm management plays a crucial role in reducing disease incidence and increasing the effectiveness of preventive interventions.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

Author Contributions

S.A.S. and AR.B. designed the study; SA.G. & F.Z.T. A.S. conducted the experimental work; S.A.S. analyzed the data; S.A.S., MH.F.M., and Z.T. wrote the manuscript, and all authors contributed to the editing the article, which was approved as a final draft.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

The study was approved by the Ethics Committee of Tehran University, Tehran, Iran.

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