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Comparative Efficacy of three Newcastle Disease Vaccine Strain in Layers

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Abstract
The aim of this study was to compare the efficacy of three most commonly used Newcastle Disease vaccines strains in layers. The Present study was conducted in the Poultry farm The University of Agriculture Peshawar. Before launching the experiment, cleaning and disinfection of cages and shed was done according to standard protocol. Before starting the experiment all the birds were screened by HI test and diseased birds were culled. The birds were screened for the existent of Newcastle disease antibodies. The birds showing nonspecific antibody titers (≤2) in HI were selected. The birds were divided into four major groups i.e. group LS, AV, MK, and Cont i.e. vaccinated with lasota (Hilton Company) VG/GA (Avinew Merial Company) Mukhtaswar (Veterinary Research Institute Peshawar) and control respectively. All birds were kept under strict observation and were checked daily at the time of feeding and watering. All the mentioned ND vaccines were purchased from farm and were administered to the birds at dose of 0.5cc subcutaneously wing. The antibody titer was determined by hemagglutination inhibition (HI) test.1 ml Blood samples were collected for the determination of antibody titer. Blood and eggs samples were collected on the day of vaccination and with week interval post vaccination till day 42. Significant difference was found among all vaccinated groups as compared to control. Significantly higher immune response was observed in group that was vaccinated with Mukhteswar strain vaccine against the Newcastle disease. Among all the vaccines of different strains, it was found that after primary vaccination chickens of the group vaccinated with Mukhtaswar strain+vitamin E+selinium produced higher immune response than the chickens of other groups vaccinated with lasota or Avineue strains. Mukhtaswar vaccine showed high response towards transmission from body to eggs. As time elapsed the titer of all vaccine gradually decreases. Birds vaccinated with Mukhtaswar + vitmin E+selinium vaccine showed significantly increase in antibody. The results showed that pullets vaccinated with Mukhtaswar+Vitamin E+Selinium vaccine was observed to have higher egg production as compared to other vaccinated and control groups. It was concluded from the present study that Mukhtaswar vaccine either individually or with supplementation significantly increase the immune response and no effect on egg production

Keywords: Growers, Pullets, Disposed Fishes, Concentrates, sun-dry fish, Vaccine strain S, Haemagglutination, Antibody titer, Haemagglutination inhibition.

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Introduction

Newcastle disease (ND) is one of the most contagious and devastating diseases to poultry in the world [1]. It is a viral disease of birds with a wide range of clinical signs from mild to severe. This disease is caused by a diverse group of viruses; the misogynic strains are endemic in Khyber Pukhtunkhwa province of Pakistan, while highly virulent strains are exotic. The highly virulent form of Newcastle disease is one of the most important poultry diseases worldwide. Chickens are particularly susceptible, and may experience high rate of morbidity and mortality up to 100%. Newcastle disease has a tremendous impact on backyard chickens in developing countries, where these birds are a significant source of protein. In developed countries, where the more virulent forms of the virus have been eradicated, trade embargoes and restrictions cause significant economic losses during outbreaks. Low pathogenicity isolates, which are common in poultry worldwide, can decrease productivity but have no impact on international trade. LaSota vaccine strain which belonged to genotype II has been widely used in China and other countries for over 40 years. However, infections of genotype VIId NDVs have still repeatedly occurred in China and other Asian countries [2-8].

Although the most significant impact of Newcastle disease is on chickens, other species can also be affected. Some pet and zoo birds become ill after infection, while other species can carry and shed virulent viruses asymptomatically. These birds, particularly illegally imported psittacines, can introduce

Newcastle disease viruses to disease free countries. Newcastle disease is also an important cause of death during the first three months of life in cormorant colonies.

Vaccination against NDV is used all over the world and the practice is adequately controlled and used by the poultry industry [9, 10]. In Pakistan, the birds are generally vaccinated initially with a mild virus LaSota or Mukteswar and then by individual application of the Mukteswar or Komarov strain. These mild or attenuated live virus vaccines can still induce a mild respiratory disease which can, in some instances, be complicated by secondary infection so their use does not constitute the ideal form of control. This incidence of failure has increased in recent years and many outbreaks have been observed. These outbreaks despite of vaccination may be either due to the failure of the existing strains of vaccines to produce the protective antibodies titer or these strains may have lost their cross immunity. Possibilities of lower virus titer in the vaccines due to exposure to heat shock may also be the contributing factor.

Materials and methods

Present study on the evaluation of Newcastle Disease vaccines was conducted in the University Poultry Farm, University of Agriculture Peshawar Pakistan. Before purchasing the experimental birds, cages and shed were cleaned and disinfected adopting standard disinfection protocols. Drinkers and feeder were disinfected with 12 % Virkon (formalin and KMNO4) solution. The shed was fumigated and kept closed till the arrival of birds. Total 96 White leg horn layers at 30 week were purchased from the local market. These birds were kept in cages under similar managemental conditions. Commercial layers ration @ 120 gm per bird was provided to all birds during the whole experimental period. The study was continued for a period of six weeks with one week adaptation period. Before starting the experiment all the birds were screened for the Newcastle disease antibodies titer using Heamaglutination Inhibition (HI) Test.

The birds were screened for the Newcastle disease antibodies according to the standard protocol as mentioned in OIE Terrestrial Manual (2008).

All the Experimental birds were divided into four groups namely A, B, C and D, having 24 birds each group. Birds in group A were vaccinated with Lasota (Hilton medion bandung-Indonesia) while birds in group B and C were vaccinated with VG/GA (Avinew Marial 29 avenue tony Garnier 69007 lyon – France), and Mukhtaswar strains (Veterinary Research Institute, Peshawar), respectively. Birds in group D were maintained as non vaccinated control. All vaccines were administered at dose rate 0.5 cc subcutaneously. Each group was further divided into two sub groups, layers in one sub group were provided with vitamin E and selenium (Nawan Company 136, sector 15'Korangi Industrial Area Karachi Pakistan) at the dose rate of one ml per 10 lit of water, while the other sub groups of each group were maintained as control for Vitamin E and Selenium. Blood and eggs samples 10 each from all birds were collected on the day of vaccination and with a week interval post vaccination till day 42. Blood samples were centrifuged at 4000 rpm for 15 minutes to collect the serum. The serum and egg yolk samples were subjected to HI test for the determination of Antibody titer against Newcastle disease. Five ml chicken blood was collected in a test tube containing EDTA by slaughtering a broiler chicks after blood collection, the blood and anticoagulant were properly mixed with EDTA gently shaken to prevent the clotting of blood. The blood was tube centrifuged at 1500 rpm for 10 minutes. The supernatant were discarded and sediment RBCs were re-suspended in 0.85% normal saline (pH 7.2).The suspension was centrifuged again at 4000 rpm for 2 minutes and the supernatant was discarded while the sediment was re-suspended in the saline solution. The washed RBCs thus prepared were stored in refrigerator at 40C. And used in one percent solution washed R.B.C solution.

Haemagglutination (HA) test was performed in 96 well polystyrene plates having U shaped bottom. A 50 µl of the normal saline was added to all wells (1- 12 with the help of micro pipette. A 50 µl of antigen was added to first well. The antigen was serially two fold diluted from well

one to eleven and last dilution was discarded. A 50 µl of washed RBCs (0.5%) were added to all wells one to twelve. The plate was incubated at room temperature (250C) for 30 minutes and results were recorded.

A 50 µl of normal saline was added to all the wells of microtitration plate 1 to 12. 50µl test serum sample. A 50 µl was added to the first well. Test serum was serially two fold diluted with the help of multi-channel pipette up to well No. 10 and the last dilution was discarded. 50 µl of 4HA of antigen was added to each well of the plate up to well No. 11. The plates were incubated at room temperature for 30 minutes. After incubation, a 50 µl RBCs (1%) suspension was added to all the wells. The plates were agitated backward and forward and from side to side to ensure even suspension of RBCs in the mixture. The plates were kept undisturbed at room temperature until a clean pattern of HA (Lattice Formation) or HI (Button Formation) was seen. The maximum dilution of each serum sample causing complete inhibition of the Haemagglutination was the titer of antibodies. The data was analyzed with statistical package SAS (1997).

Results and discussion

Newcastle disease (ND) is one of the most important diseases of the poultry. This is epidemic throughout the world. Different vaccines are used to protect chickens from Newcastle disease throughout the world. The present study is based on the immune responses comparison of different ND-vaccines in 30 weeks old layers in term of antibody titer determination are given below.

Comparisons of HI titer in serum among different ND-vaccines

The results of antibody titers against ND virus in different groups are presented in Table 1. The HI antibody titers in vaccinated groups was significantly ($P<0.05$) higher than the control group.

Table 1: Comparative antibody titer (HI) in different vaccinated groups

Group	Comparative Antibody titer (HI)					
	Mean ±SE Week I	Mean ±SE Week II	Mean ±SE Week III	Mean ±SE Week IV	Mean ±SE Week V	Mean ±SE Over all
Lasota	149.33±5.33	192.00^{a}±9.23	618.66 ^{b}±42.6	684.00^{b}±64.0	234.66 ^{b}±5.33	375.33^{b}±5.78
Mukhtaswar	149.33±5.33	213.33^{a}±10.66	810.66 ^{a}±42.6	682.66 ^{a}±76.9	309.33^{a}±10.6	433.31^{a}±18.74
Avineue	149.33±5.33	213.33^{a}±28.22	682.66 ^{ba}±56.4	533.33^{ba}±56.4	277.33^{ba}±28.2	371.20^{b}±9.73
Control	149.33±5.33	53.33 ^{b}±2.66	16.66 ^{c} ±0.66	3.33 ^{c} ±0.33	0.00 ^{c}±0.00	044.53^{c}±3.64

*Means with different superscripts are significantly different at @0.05

Table 2: Comparative antibody titer in vitamin E-selenium supplemented groups.

Group	Comparative antibody titer with vitamin E-selenium supplementation					
	Mean ±SE Week I	Mean ±SE Week II	Mean ±SE Week III	Mean ±SE Week IV	Mean ±SE Week V	Mean ±S Over all
Lasota	149.33± 1.6	205.33^{a}± 5.3	725.33^{b}± 18.6	501.33^{b}± 7.9	253.33^{b}± 16.7	366.93^{b}±
Mukteswar	149.33± 1.6	253.33^{a} ± 8.9	938.66^{a}± 23.4	832.33^{a}± 10.6	389.33^{a}± 13.0	512.59^{a}±
Avineue	149.33± 7.7	218.66^{a} ± 5.8	746.66^{ba}±10.6	586.66^{b}± 11.6	346.66^{b}± 9.9	409.59^{b}±
Control	149.33± 7.7	56.00 ^{b} ± 2.0	17.66^{c} ± 2.23	5.00^{c}± 2.80	0.00 ^{c} ±0.00	45.59^{c}±2
ND-vaccines and ND-vaccines+ Vit -E selenium effect on HI-titer						
ND-vaccines	149.33± 1.13	165.33±7.78	540.16^{b}± 27.90	400.83^{b}± 21.92	204^{a}± 17.81	291.93^{a}±
ND vaccines +E-S	149.33± 1.13	198.66± 7.68	682.00^{a}± 34.07	561.66^{a}± 25.48	289.33^{b}± 4.70	376.20^{b}±

*Means with different superscripts are significantly different at @0.05

This indicated the effectiveness of vaccines used in this study. Among the vaccinated groups, vaccinated with Mukhtaswar vaccine had highest HI antibody titer (433.3±18.78.Birds in groups A and C were vaccinated with lasota vaccine and VgGA had statistically similar antibody titer. This indicated the better immune response of Mukhtaswar vaccine in our locality. The mentioned vaccine is locally prepared after the attenuation of local virus.

Serum HI Antibody in Response to Supplementation

Serum HI antibody titer in supplemented birds was significantly higher than the non-supplemented layers (Table 2). Vaccinated groups showed higher response in birds vaccinated with Mukhtaswar followed by the birds vaccinated with lasota and VgGA. Muktaswar vaccine in combine state with supplementation showed maximum protection level against ND disease by showing higher immune response as compared to other vaccine either individual or with supplementation. This indicated that Vitamin E and Selenium could be also used for enhancing the immunity of birds by improving the activities of antibodies production after vaccination.

HI- Titer Comparison among different ND-vaccines from Egg yolk

Layers were vaccinated with ND-lasota, ND-Mukhtaswar and ND-Avineue vaccine through sub cut route and the antibody titer determined from egg yolk are presented in Table 3. It was observed that the antibody transmission through egg yolk of all vaccines showed significantly ($P<0.05$) high level than the control. While among the vaccine Mukhtaswer vaccine showed high response towards transmission form body to eggs. As time elapsed the titer of all vaccine gradually decreased.

Table 3: Egg Yolk HI- Antibody Titer of Different ND-vaccines

	Comparative Antibody titer (HI) of egg yolk					
Group	**Mean ±SE Week I**	**Mean ±SE Week II**	**Mean ±SE Week III**	**Mean ±SE Week IV**	**Mean ±SE Week V**	**Mean ±SE Over all**
Lasota	53.33±2.66	90.66^{a}±20.34	213.33^{b}±8.66	138.66^{b}±13.32	112.00^{b}±14.28	121.20^{b}±19.86
Mukhtaswar	53.33±2.66	96.00^{a}±28.86	266.66^{a}±18.33	202.66^{a} ±9.11	149.33^{a} ±5.33	153.60^{a}±14.76
Avineue	53.33±2.66	90.66^{a}±10.66	224.00^{ba}±0.00	170.66^{ba}±10.66	133.33^{a} ±5.33	138.40^{b}±46.07
Control	53.33±2.66	45.33^{b} ±2.66	32.00^{c}±4.00	14.00^{c}±1.15	0.00^{c}±0.00	29.03^{c}±13.76

*Means with different superscripts are significantly different at @0.05

Table 4: Comparison of HI- antibody titer between supplemented and control groups

	Comparative antibody titer of egg yolk with vitamin E-selenium supplementation					
Group	**Mean ±SE Week I**	**Mean ±SE Week II**	**Mean ±SE Week III**	**Mean ±SE Week IV**	**Mean ±SE Week V**	**Mean ±SE Over all**
Lasota	64.66±21.0	154.6^{b}±30.2	507.3^{b}±126.7	378.66^{b}±71.3	192.0^{b}±48.0	259.4^{b}±27.9
Mukteswar	65.00±21.3	205.6^{ba}±44.7	768.3^{a}±202.3	592.00^{a}±175.2	312.0^{a}±62.6	380.6^{a}±53.8
Avineue	64.66±6.4	194.3^{a}±24.9	713.0^{a}±46.7	565.33^{a} ±30.5	298.6^{a}±72.6	367.2^{b}±32.5
Control	64.33 ±3.3	56.00^{c}±2.06	17.66^{c}±0.61	5.00^{c}±0.96	0.00^{c} ±0.00	28.59^{c} ±21.8
ND-vaccines and ND-vaccines+ Vit -E selenium effect on HI-titer						
ND-vaccines	64.66±13.4	105.3^{b}±16.8	329.8^{b}±89.1	219.50^{b}±60.0	117.3^{b}±24.5	167.3^{b}±57.5
ND vaccines +E-S	64.66 ±03.6	200.0^{a}±27.3	673.3^{a}±126.6	551.00^{a}±106.5	284.0^{a}±58.1	354.6^{a}±102.3

*Means with different superscripts are significantly different at @0.05

Table 5: Average egg production of layers vaccinated with different ND-vaccine

	Egg production of layers					
Group	**Mean ±SE Week I**	**Mean ±SE Week II**	**Mean ±SE Week III**	**Mean ±SE Week IV**	**Mean ±SE Week V**	**Mean ±SE Over all**
Lasota	3.00±0.00	3.33^{a}±0.33	3.33^{a} ±0.33	3.33^{a} ±0.66	2.66^{a} ±0.66	3.13^{a}±1.33
Mukhtaswar	3.00±0.00	3.33^{a}±0.33	3.66^{a} ±0.33	3.66^{a} ±0.33	3.33^{a} ±0.33	3.39^{a}±0.23
Avineue	3.00±0.00	3.33^{a}±0.33	3.33^{a} ±0.33	3.66^{a} ±0.33	3.00^{a} ±0.00	3.26^{a}±1.34
Control	3.00±0.00	2.66^{b} ±0.33	2.00^{b} ±0.00	0.33^{b} ±0.33	0.00^{b} ±0.00	1.58^{b}±0.43

*Means with different superscripts are significantly different at @0.05

Egg yolk HI- antibody titer of different ND-vaccines in response to supplementation

Table No 4 shows that the egg yolk HI antibody was maximum in supplemented

Layers ($P<0.05$). Among the vaccinated groups the effect of supplementation of birds vaccinated with Mukhtaswar vaccine was maximum as compared to the birds vaccinated with lasota and VgGA. The difference in immune response of the layers vaccinated individually and with supplementation might be due to the Selenium which act as a constituent of cytosolic enzyme glutathione peroxidase convert

free radicals to inert substances rendering them harmless).

Comparison of egg production in layers immunized with different Newcastle disease-vaccines

The weekly egg production was calculated and is presented in Tables 5 and 6. The results indicated that layers vaccinated with Mukteswar+ supplementation showed higher egg production as compared to other vaccinated and control group. Result from first two weeks showed that the all vaccine used in the experiment had same effect on the egg production patron. But the result of remaining three weeks indicated that the Mukhtaswar strain had significant ($P<0.05$) effect on egg production as compared to other vaccinated and non-vaccinated groups. The combine state of vaccine and supplementation resulted low egg production then that of individual vaccine practices and to the control group.This indicate that supplementation with vaccine did not improve the egg production in experimental birds.

Correlation between the HI titer in Serum and Egg yolk

It is clear that there is a high degree of correlation between serum and egg antibody titers. It shows that as the antibody titer increases in serum the egg yolk antibody titer also increases However, serum HI titer was considerably higher than HI titers in egg.

Newcastle disease (ND) is one of the most important diseases of the poultry. This is epidemic throughout the world. Different vaccines are used to protect chickens from Newcastle disease throughout the world. The present study is based on the immune responses comparison of different ND-vaccines in 30 weeks old layers in term of antibody titer determination are given below.

Table 6: Effect of different ND-vaccine and ND-vaccine + supplementation on egg production

Group	Egg production of layers with vitamin E-selenium supplementation					
	Mean ±SE Week I	Mean ±SE Week II	Mean ±SE Week III	Mean ±SE Week IV	Mean ±SE Week V	Mean ±SE Over all
Lasota	3.00±0.00	3.33±0.21	3.33^{a}±0.21	3.50^{a} ±0.22	2.83^{b} ±0.16	3.19^{a}±0.23
Mukteswar	3.00±0.00	3.33±0.21	3.66^{a} ±0.21	3.66^{a} ±0.21	3.33^{a} ±0.21	3.36^{a}±0.13
Avineue	3.00±0.00	3.33±0.21	3.50^{a} ±0.22	3.50^{a} ±0.22	3.00^{ba} ±0.00	3.20^{a}±0.54
Control	3.00±0.00	2.83±0.16	2.16^{b} ±0.16	0.66^{b} ±0.21	0.66^{c} ±0.21	1.86^{b}±0.32
ND-vaccines and ND-vaccines+ Vit -E selenium effect on HI-titer						
ND-vaccines	3.00±0.00	3.16±0.16	3.08±0.22	2.83±0.45	2.25±0.41	2.90±0.64
ND vaccines +E-S	3.00±0.00	2.25±0.13	2.25±0.21	2.83±0.34	2.14±0.37	2.50±087

*Means with different superscripts are significantly different at @0.05

Comparisons of HI titer in serum among different ND-vaccines

The results of antibody titers against ND virus in different groups are presented in Table. The HI antibody titer in vaccinated groups was significantly ($P<0.05$) higher than the control group. This indicated the effectiveness of vaccines used in this study. Among the vaccinated groups, vaccinated with Mukhtaswar vaccine had highest HI antibody titer (433.3±18.78.Birds in groups A and C were birds were vaccinated with lasota vaccine and VgGA had statistically similar antibody titer. This indicated the better immune response of Mukhtaswar vaccine in our locality. The mentioned vaccine is locally prepared after the attenuation of local virus.

Serum HI Antibody in Response to Supplementation

Serum HI antibody titer in supplemented birds was significantly higher than the non supplemented layers (Table 2). Vaccinated groups showed higher response in birds vaccinated with Mukhtaswar followed by the birds vaccinated with losta and VgGA (fig.1). Mukteswar vaccine in combine state with supplementation showed maximum protection level against ND disease by showing higher immune response as compared to other vaccine either individual or with supplementation. This indicated that Vitamin E and Selenium could be also used for enhancing the immunity of birds by improving the activities of antibodies production after vaccination.

HI- Titer Comparison among Different ND-vaccines from Egg yolk

Layers were vaccinated with ND-lasota, ND-Mukhtaswer and ND-Avineue vaccine through subcut route and the antibody titer determined from egg yolk are presented in Table 3. It was observed that the antibody transmission through egg yolk of all vaccines showed significantly ($P<0.05$) high level than the control. While among the vaccine Mukhtaswer vaccine showed high response towards transmission form body to eggs. As time elapsed the titer of all vaccine gradually decreased.

Egg Yolk HI- antibody titer of different ND-vaccines in response to supplementation:

Table No 4 hows that the egg yolk HI antibody was maximum in supplemented layers ($P<0.05$). Among the vaccinated groups the effect of supplementation of birds vaccinated with Mukhtaswar vaccine was maximum as compared to the birds vaccinated with lasota and VgGA. (Fig.2). The difference in immune response of the layers vaccinated individually and with supplementation might be due to the Selenium which act as a constituent of cytosolic enzyme glutathione peroxidase convert free radicals to inert substances rendering them harmless. Kanchana and Jeyanthi [11] reported that combination of vaccine and Vimsel supplementation significantly improved both humoral and cell mediated immunity in birds. Other researchers also report significantly higher antibody titers (HI and ELISA) against NDV in broilers fed with diets receiving 0.06 mg/kg selenium [12, 13]. The reason for the contradiction among the results of present study and the findings of Boa-Amponsem et al. [14] might be the result of the difference in the species used, age of birds and the amount of vitamin E and selenium supplemented to the diet.

Comparison of egg production in layers immunized with different Newcastle disease-vaccines

The weekly egg production was calculated and is presented in Tables 5 and 6. The results indicated that layers vaccinated with Mukteswar+supplementation showed higher egg production as compared to other vaccinated and control group. Result from first two weeks showed that the all vaccine used in the experiment had same effect on the egg production patron. But the result of remaining three weeks indicated that the Mukteswar strain had significant ($P<0.05$) effect on egg production as compared to other vaccinated and non-vaccinated groups. The combine state of vaccine and supplementation resulted low egg production then that of individual vaccine practices and to the control group (Fig.3).This indicate that supplementation with vaccine did not improve the egg production in experimental birds Rehmani et al. [15] worked on the effect of Mukteswar and Komarov strains of Newcastle Disease vaccines on egg production and found that Komarov ND vaccine demonstrated 11% less egg production than the birds vaccinated with Mukteswar ND vaccine. Hanson [16] reported that live ND vaccine produced adverse reactions along with a drop in egg production.

Conclusion

The overall conclusion of the current study was that, that Muktaswar Strains of ND vaccine was found better in respect of antibody titer and there was no effect of it on egg production, also we investigated that Vitamin E and selenium supplementation supports antibody production, furthermore the present study highlighted that there was no effect of supplementation on egg production.

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