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COMPARATIVE IMMUNOGENIC RESPONSE OF LIVE VACCINE OF LASOTA AND MUKTESWAR STRAINS OF NEWCASTLE DISEASE VIRUS BY DIFFERENT ROUTES OF INOCULATION IN LAYER CHICKEN

ABDUL WHAB MANZOOR[1*], SAJJAD HUSSAIN[1], HAFIZ MUHAMMAD WAQAR[1], SADIA SARFARAZ[1], SHERAZ SHAHID[1], SAMIULLAH[1], AFEEFA SHAFIQ[1], SAJJAD ALI[1], RAIS AHMED[2], HALEEMA SADIA[3], MAHEEN MURTAZA[4], TASLEEM KAUSAR[5]

[1]Veterinary Research Institute, Zarrar Shaheed Road, 54810, Lahore Cantt.
[2]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore.
[3]Department of Biotechnology, Balochistan University of Information Technology Engineering and Management Sciences, Quetta, Pakistan.
[4]Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur.
[5]Department of Zoology, Government Sadiq College University, 63100, Bahawalpur

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Key words:
ND-LaSota, ND-Mukteswar, Ocular, Drinking Water, Injection, Haemagglutination inhibition, Immune Response.

ABSTRACT

Newcastle disease (ND) has been one of the great challenges to Poultry in Pakistan and dealt with vaccination. Mortality and morbidity that are associated with it are economically significant. Two vaccines produced against Newcastle disease virus are Live ND-LaSota and ND Mukteswar vaccines and their immunogenic effects in backyard poultry. For that purpose, a total 130 of 6-8 weeks old layer cockerels were inoculated with both vaccines by different routes including Ocular, drinking water and Injection and serum samples were collected at 14, 21 and 35 days' post inoculation were analyzed by Heamaglutination inhibition test for antibody titers. The best route of administration for ND-LaSota was observed by ocular drops and ND- Mukteswar through injection. So it was concluded that both the vaccines needed booster doses in 4th to 5th week of primary inoculation. ND-LaSota vaccine was found to be best for primary induction.

1. INTRODUCTION

Poultry is one of the most well developed and advanced sectors of the economy of Pakistan employing almost 1.5 million citizens through direct or indirect employment. According to the Economic Survey of Pakistan 2018-19, poultry is a source of proteins for 220 million people of the country and contributes 1.4% to the national GDP (GOP, 2019). The emerging and prevalent infectious diseases pose great economic losses along with serious threat to the growth of rural as well as commercial poultry sector (Aalexander, 2003). These infectious diseases like Avian Infectious bronchitis (IB), Avian Influenza (AI), Newcastle disease (ND), Infectious Bursal disease (IBD) etc., are responsible for huge economic losses in the form of mortality, therapeutic expenses, cost of preventive measures and restrictions in exports of animal and poultry (Absalón et al., 2019).

One of the most notorious infectious diseases is Newcastle disease caused by Avian Paramyxovirus1 (APMV1) and characterized by various forms. Its viscerotropic form causes sudden and high mortality with intestinal hemorrhages while neurotropic form is associated with severe respiratory and nervous signs (El-Masry et al., 2019). The most important method of controlling ND is vaccination, accompanied by strict biosecurity measures and quarantine policy (Bello et al., 2018). Vaccine is prepared in different forms from lentogenic and mesogenic strains of ND virus e.g LaSota, Mukteswer, B1, V4, Komarov. Among these strains LaSota and Mukteswer are mostly used for vaccination development against ND in Pakistan (Rasool et al., 2021). LaSota is a lentogenic strain of the virus used for chicks less than 8 weeks of age in Pakistan. According to some studies, LaSota strain causes vaccination reaction, hence not recommended for naïve birds as well as mixed population of different aged flocks (Abel, 2018). Mesogenic strains (Mukteswer) are not recommended in

*Corresponding Author: whab2010@gmail.com

the chickens less than 8 weeks of age as they may cause respiratory distress and mild enteric disease and may cause mortality in immuno-compromised chickens. Mesogenic strains provide good immune response long lasting immunity and are usually used to boost the primary titer produced by Lentogenic strains (Zhao et al., 2012). ND viruses are constantly evolving but all types are considered to be same serological type and antibody produced by one vaccinal or pathogenic strain provides cross protection against all other strains (Bello et al., 2018).

In Pakistan, ND vaccines are produced from two strains (ND La Sota and ND Mukteswer) at veterinary Research Institute (VRI), Lahore which is a public sector institution. Vaccines produced are usually used for backyard poultry and small-scale farmers. The commercial poultry companies import ND vaccines from other countries. There was a need to compare the immunogenic efficacy of these two vaccines, inoculated by different routes including ocular drops, injection and drinking water, through serology. The immunogenic response following ocular drops of ND-LaSota and booster by injecting ND-Mukteswer vaccine was compared to determine the boosting effect of the vaccine. Therefore, this project was aimed to study the comparative efficacy of live vaccine of both strains with respect to immunogenic response followed by recommendations to the industry and backyard farmers in Pakistan about the comparative suitability of both vaccines for onward vaccine production in Pakistan.

2. MATERIALS AND METHODS

ND Vaccines (ND-LaSota & ND-Mukteswar) produced at VRI, Lahore were used for the current study. These vaccines were produced on 9-11 days old embryonated chicken eggs, received from poultry unit at VRI, Lahore, disinfected and incubated at 37ºC. The eggs were inoculated at 9th to10th day of incubation with 0.1 ml of 105-106 dilution of virus and were incubated for another 96 hours for La Sota and till the death of embryo in case of Mukteswar strain. Then eggs were chilled and harvested. The allantoic fluid was harvested in case of LaSota strain but both fluid and embryo were collected in case of Mukteswer strain. Vaccines were lyophilized by freezing at –35ºC, primary drying by gradually raising the temperature from -25ºC to Zero degree in 18 hours at 170 micro bar and secondary drying at +35ºC for 10 hours. The vials were then sealed and tested for presence of vacuum. Each vial contained 200 doses and quality was assured by safety, sterility, EID50 and ELD50.

The experiment was conducted on 130, day-old layer chicks which were purchased from the market and were reared at VRI, Lahore up to 06-08 weeks and were divided into three groups (01, 02, 03). The group 01 and 02 contained forty birds, each group was further divided into four subgroups (A, B, C, D) containing 10 birds each. The group 03 consisted of 50 birds and further divided into three subgroups (A, B and C) with group A and B having 20 birds each and group C with 10 birds as control. All the birds were kept under normal conditions in cages in experimental sheds of Veterinary Research Institute Lahore, Pakistan and study was conducted under legal and ethical rules of Government of the Punjab (Banu, 2009). All the birds were screened for antibody titer by haemagglutination inhibition (HI) assay. The birds with high antibody titer were replaced (Anon, 1971).

Group-1

The birds of subgroup A, B and C were inoculated with ND-LaSota Live vaccine @) 0.1ml/ bird by ocular drops, drinking water and intramuscular injection, respectively while the birds of group D were kept as control. The doses were calculated as107.85 EID50/dose. The antibody titer of all the birds in all sampling units of group 01 were checked by HI test after 14, 21 and 35days post-inoculation.

Group-2

Protocol adopted for group 01 was repeated using ND-Mukteswar vaccine. The doses were calculated as 107.20 ELD50/dose.

Group-3

This group of 50 birds was vaccinated with ND Live LaSota and ND Mukteswar live vaccine. In Subgroup A, 20 birds were vaccinated with ND-LaSota live Vaccine at day 01 by ocular route at dose rate of 0.1ml /bird while the birds of subgroup B were vaccinated with ND- LaSota live Vaccine at day 01 by Drinking water. Both groups were given a booster dose using 0.1ml intramuscular injection of ND- Mukteswar live vaccine 14 days after the first injection. The antibody titers of both sub-groups were measured with HI test after 14, 21 and 35 days after 2nd injection. Subgroup C with 10 birds was kept as control (Table 1).

Data was analyzed by ANOVA using SPSS (V 20.0). Statistical significance was defined as a value of $p<0.05$. Paired‘t’ test were performed to determine the significant differences in HI titres of chickens of a group after primary and secondary vaccination (Beri, 2005).

3. RESULTS AND DISCUSSION

ND-LaSota Vaccine

The titers of Sub-group A, inoculated by ocular drops of ND-LaSota (SGA-L) were considerably higher than other subgroups. Drinking water proved to be second best route of administration of LaSota vaccine. The titer of Subgroup B, administered through drinking water (SGB-

L) and subgroup C, inoculated through injection (SGC-L) remained higher than SGA-L at the end of experiment. The antibody response on inoculation by injection was poor when compared to other routes. Moreover, the antibody titers of all groups decreased in the 4th to 5th week post inoculation suggesting the booster dose in 5th week of first inoculation (Figure 1).

Table 1. Vaccine Inoculation Layout in Different Groups.

Group 01 (ND-LaSota)	Subgroup A	ND-LaSota live vaccine ocular drops 0.1 ml/bird
	Subgroup B	ND-LaSota live vaccine drinking water 0.1 ml/bird
	Subgroup C	ND-LaSota live vaccine Injection Intramuscular (I/M) 0.1 ml/bird
	Subgroup D	Control group
Group 02 (ND-Mukteswar)	Subgroup A	ND-Mukteswar live vaccine ocular drops 0.1 ml/bird
	Subgroup B	ND-Mukteswar live vaccine drinking water 0.1 ml/bird
	Subgroup C	ND-Mukteswar live vaccine Injection (I/M) 0.1 ml/bird
	Subgroup D	Control group
Group 03 (ND-LaSota + ND-Mukteswar)	Subgroup A	ND-LaSota live vaccine ocular drops and booster by ND-Mukteswar live vaccine Injection
	Subgroup B	ND-LaSota live vaccine Drinking water and booster by ND- Mukteswar live vaccine Injection
	Subgroup C	Control group

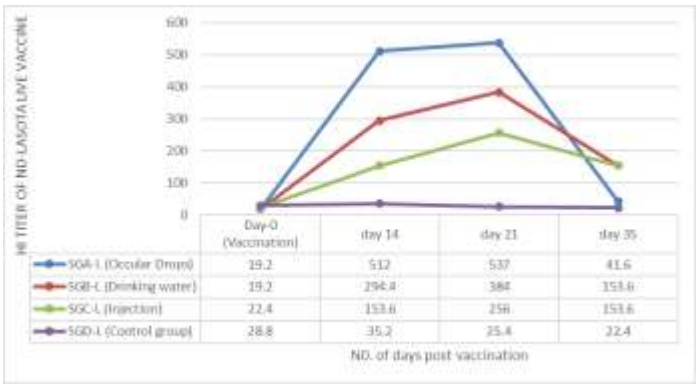

Figure 1: Antibody titre of ND-LASOTA live vaccine by different routes of inoculation

ND-Mukteswar Vaccine

The titer of all the groups were higher than control. Subgroup A, inoculated with ND- Mukteswar vaccine by ocular drops (SGA-M) showed poor titers as compared to other groups. Also, eye drops of Mukteswar vaccine caused redness of eyes in birds. The titers appeared late in SGA-M and remained same even after 35th day of inoculation. The titer of subgroup C, inoculated by injection (SGC-M) showed best results in group 02, suggesting the injection as best route of administration for ND- Mukteswar Vaccine. Inoculation by drinking water (SGB-M) showed intermediate response. Also, the titers of SGB-M and SGC-M decreased in the 5th week of initial inoculation suggesting the need of booster dose in the 5th week (Figure 2).

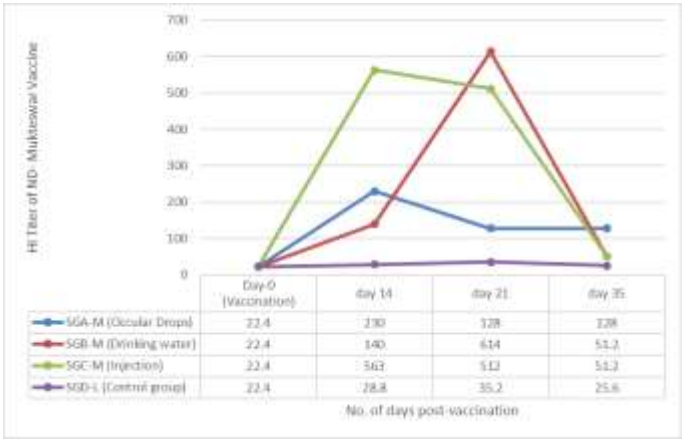

Figure 2: Antibody titre of ND- MUKTESWAR live vaccine by different routes of inoculation

Combination of ND-LaSota and ND-Mukteswar Vaccine

The inoculation of ND- Mukteswar vaccine as a booster following the ND-LaSota vaccine (SGA-LM and SGB-LM) showed considerable high titers even after 35 days of injection suggesting that ND-LaSota primary inoculation should be boosted with ND-Mukteswar vaccine by injection in 3rd to 4th week of primary inoculation (Figure 3).

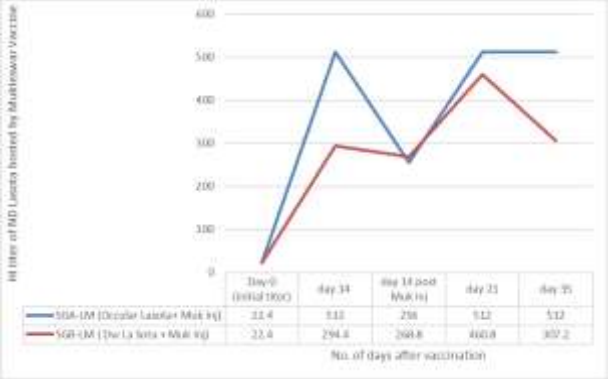

Figure 3: Antibody titre of ND LASOTA vaccine boosted by ND MUKTESWAR injection.

Comparative analysis of ND LaSota and Mukteswer Vaccines

All the routes of ND- LaSota and Muktesawer vaccines were compared on the basis of experimental data. The results showed that the inoculation by ocular drops proved to be best route of LaSota vaccine and worst for Mukteswar vaccine (Figure 4). The drinking water showed intermediate response as compared to other routes for both vaccines. Mukteswar vaccine performed better in drinking water as compared to Lasota (Figure 5). The injection proved to be best route of administration for ND-Mukteswar vaccine and worst for ND-LaSota (Figure 6). Both vaccines performed almost equally well when administered by their best route of inoculation i.e Occular drops for ND-LaSota and injection by ND-Mukteswar in 6-8 week old layer chicken (Figure 7).

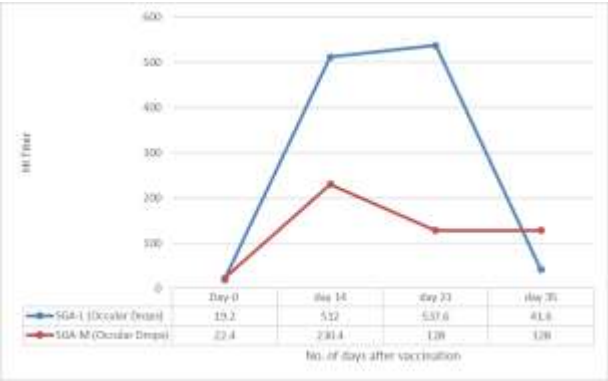

Figure 4: Comparison of ND-LASOTA and MUKTESWAR live vaccines by occular route

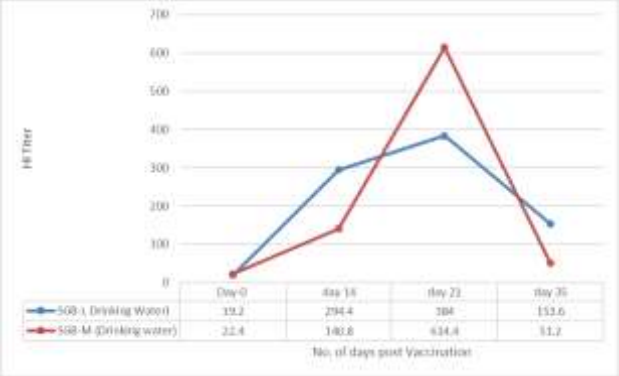

Figure 5: Comparison of ND-LASOTA and MUKTESWAR live vaccines by drinking water

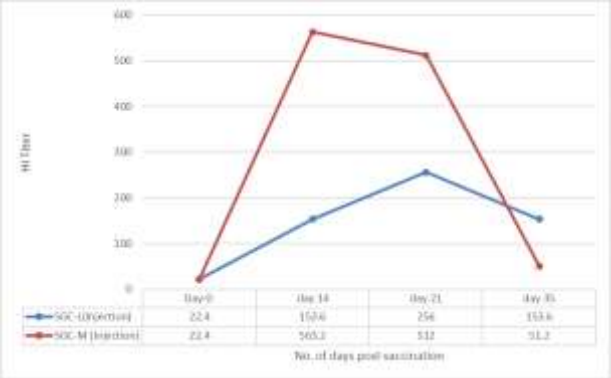

Figure 6: Comparison of ND-LASOTA and MUKTESWAR live vaccines by injection

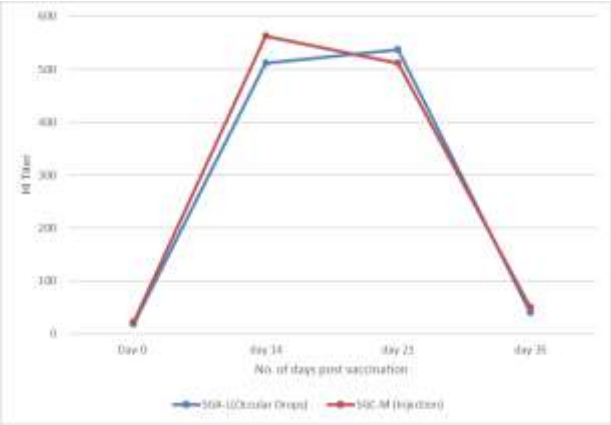

Figure 7: Comparison of ND-LASOTA (Occular Drops) and ND- MUKTESWAR (INJECTION)

Data was analyzed by ANOVA using SPSS (V 20.0). ND Lasota given to birds in three different routes (ocular, oral and injection) showed a significant difference ($P<0.05$, df=3, $F=5.759$). Analysis through post hoc test (LSD)

showed more significance in ocular route (P<0.05) (Table 2). Mukteswar strain given to birds in three different routes (ocular, drinking and injection) showed a significant difference (P<0.05, df=3, F=5.874). Analysis through post hoc test (LSD) showed more significance in injection route (P<0.05) (Table 2).

Data was analyzed by paired sample t- test using SPSS (v 20.0). Vaccine type (Lasota & Mukteswar) have statistically significant difference t (49) = -11.068, p=0.001. R value (0.949) indicates strong positive correlation between two vaccine types and also indicates strong significance for selection of vaccine route (Mean= -1.000, Std. Error= 0.090). Route of vaccination (Oral, Drinking & Injection)) and antibody titer have statistically significant difference t (49) = -9.431, p=0.001. R value (0.334) indicates strong positive correlation between Route of vaccination and antibody titer. This association also indicates strong significance for selection of vaccine route for good antibody titer in birds and very high significance of antibody titer than vaccine route (Mean= -304.84000, Std. Error= 32.324). Data was analyzed by paired sample t- test using SPSS (v 20.0). Route of vaccination (Oral, Drinking & Injection) and antibody titer have statistically significant difference t (49) = -9.431, p=0.001. R value (0.334) indicates strong positive correlation between Route of vaccination and antibody titer. This association also indicates strong significance for selection of vaccine route for good antibody titer in birds and very high significance of antibody titer than vaccine route (Mean= -304.84000, Std. Error= 32.324).

Newcastle disease has caused valuable damage to the poultry industry in Pakistan. For effective control of the disease, vaccination of the birds plays an important role. The indigenous vaccine production in Pakistan is minimal and most of the vaccines are imported. The vaccines produced against Newcastle disease virus at Veterinary Research Institute, Lahore are Live ND-LaSota and ND Mukteswar vaccines. The route of administration of the vaccine is equally important to protect the birds from the disease. A study was needed to determine the best route of administration of these vaccines.

The current study was aimed to discover the route of administration of the ND vaccines to get higher titers for effective control of the disease. Different routes of inoculation including ocular drops, drinking water and intramuscular injection were applied for ND-LaSota and ND-Mukteswar vaccine. The higher titer was observed with ND-LaSota vaccine inoculated by ocular drops. Rehmani (1996) reported similar findings as ND-LaSota vaccine administered intraocularly ranked the best. Similar findings were observed by Wegdan et al. (2015) and Hassanzadeh et al. (2020) where they found higher titers of ND vaccine when administered through intraocular route.

Low antibody titer was observed in case of vaccine inoculation through intramuscular injection for ND-LaSota vaccine while high HI titer was observed by intramuscular injection of ND-Mukteswar vaccine. The antibody response of Mukteswar vaccine through drinking water was found to be lower as compared to intramuscular injection route. Rehmani (1996) also reported low titer of Mukteswar vaccine when administered through drinking water.

HI Titer of both the vaccines dropped in the 4th to 5th week post inoculation. Similar findings were recorded by Sanda et al. (2015) where immunity was found low below the protective level in the 5th week post vaccination. Hence booster dose may be administered in 4th to 5th week post vaccination. A more recent developments in vaccines have been initiated against a wider range of viruses affecting different animals. These include lyophilized live Newcastle Disease (LaSota) vaccine (Khatoon et al., 2022) bovine Herpesvirus 1 gE Deleted Vaccine (Rehman et al., 2022) against newly isolated strains of herpesvirus in cattle from Pakistan (Rehman et al., 2021), and Multiepitope-based Subunit Vaccine (MESV) against foot and mouth disease virus. The efforts to save livestock and poultry from emerging and mutating pathogens in the study area through vaccine is need of the hour (Riaz et al., 2021).

4. CONCLUSION

Inoculation by ocular drops proved to be best route of LaSota vaccine and worst for Mukteswar vaccine. The drinking water showed intermediate response. Mukteswar vaccine performed better in drinking water. The injection proved to be best route of administration for ND-Mukteswar vaccine and worst for ND-LaSota. Both vaccines performed almost equally well when administered by their best route of inoculation i.e. Occular drops for ND-LaSota and injection by ND-Mukteswar in 6-8 week old layer chicken and both need booster injection in 4th to 5th week post vaccination.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 2 Antibody titre of Lasota vaccine and Mukteswar vaccine at different route of inoculation

Routes	Lasota given to group 1 (SGA-SGD) in three different routes	Mukteswar given to group 2 (SGA-SGD) in three different routes
	Mean± Std. Deviation	**Mean± Std. Deviation**
SGA (Ocular)	277.60 ± 293.21^{a}	127.20 ± 107.58^{ac}
SGB (Drinking)	212.80 ± 251.06^{ab}	207.20 ± 269.56^{ab}
SGC (Injection)	146.40 ± 92.90^{bc}	287.20 ± 288.26^{b}
SGD (Control)	28.00 ± 11.46^{cd}	28.00 ± 11.46^{C}

Different superscript within column indicate significant difference ($p<0.05$).
SGA= sub group A, SGB= sub group B, SGC= sub group C, SGD= sub group D.