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Research Article

Evaluation of Two Vaccination Schemes Using Live Vaccines against Newcastle Disease in Chickens

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Abstract: The use of different types of Newcastle disease (ND) vaccine in different vaccination schemes has decreased the incidence of velogenic Newcastle disease (VND) in commercial poultry worldwide. In under-developed countries like Pakistan, these vaccination schemes are not successful due to free-range and backyard poultry production.

This trial was conducted in 90 experimental chickens to develop an effective control against ND. The level of antibody response, detected by haemagglutination-inhibition (HI) test, and the degree of protection against the virulent strain of Newcastle disease virus (NDV) were studied. The chickens were immunized with commercially available ND vaccines. In scheme A, primary vaccination was done with La Sota vaccine with 10^9 EID_{50}, administered on day 5 by eye drop (E/D), followed by a booster vaccination with the same vaccine and the same route on day 21 and, in scheme B, primary vaccination was done with the same vaccine (La Sota vaccine with 10^9 EID_{50}), administered on day 5 by E/D, followed by a booster vaccination with a mesogenic strain (Mukteshwar) given intramuscularly on day 21.

Both schemes of immunization conducted ensure comparatively solid immunity when challenged with a virulent field isolate of ND at 6 weeks of age. A better protection index was obtained from chickens vaccinated with scheme B.

Key Words: Newcastle disease, ND, La Sota, mesogenic, Mukteshwar, live vaccines, immunization, chicken

Introduction

Newcastle disease (ND) is one of the most infectious, highly contagious, fatal viral diseases of chickens, characterized by respiratory, digestive, and nervous symptoms as reported by Mishra et al. (1). According to Gallili and Ben-Nathan (2), it is a worldwide problem with severe economic implications, affecting chickens, turkeys, and other birds. Alexander (3) stated that the morbidity and mortality of susceptible birds may reach up to 100% in the severe form of the disease, and unvaccinated birds are more prone to the disease. Biosecurity and vaccination are 2 important measures to address the problem and have been used successfully for its prevention and control for a long time as mentioned by Glisson and Kleven (4). Chandraseker et al. (5) reported that vaccination protects the birds by producing humoral and cell-mediated immune responses. Both these responses are essential for complete protection against infections. In chickens, live vaccines administered by eye drop or orally have been found to induce protective mucosal immunity mediated by immunoglobulin (Ig)A antibodies as reported by Jayawardane and Spradbrow (6), and Parry and Aitken (7). On the other hand, Folitse et al. (8) found that injected inactivated vaccines led to the production of high levels of serum antibodies producing humoral immunity that will protect the chicken against infection with the virus. According to Van Eck (9), the disadvantage of inactivated vaccines over live vaccines is that inactivated oil emulsion vaccines do not induce local immunity in the respiratory and digestive tracts; however, immunity is established rather slowly. Killed vaccines are expensive and difficult to administer than live vaccines.

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Many trials have been conducted to develop a single annual vaccination program that can significantly control ND and reduce the vaccination cost. In Pakistan various vaccines are available commercially for the control of ND. The objective of this study was to develop a vaccination program that will improve the antibody response and will give good protection against challenge with virulent virus. The basic hypothesis of this study was that the efficacy of a lentogenic strain (La Sota) vaccine could be improved by subsequent administration of a mesogenic strain (Mukteshwer) vaccine.

Materials and Methods

Chickens

One-day-old unvaccinated chicks were procured from a local hatchery. They were brooded together for 5 days in a disease-free animal house, until they were divided into 3 groups. The unvaccinated control group was kept in the same building where vaccinated birds were kept. The birds received appropriate feeding and husbandry throughout the experimental period.

Commercial Vaccines

A La Sota virus vaccine manufactured commercially (Table 1) and a mesogenic vaccine (Mukteswar strain) produced locally were procured. They were administered according to the manufacturers' instructions.

Challenge virus

A virulent field isolate of velogenic Newcastle disease (VND) was selected that was previously characterized as velogenic by the research workers in the Department of Microbiology, University of Veterinary and Animal Sciences, Lahore. Concentration of the virus was $10^{5.5}$ LD_{50} and the challenge dose was 0.1 ml ($100 \times LD_{50}$) per bird.

Experimental design

The experimental design is given in Table 1. The experiment lasted 62 days. On day 1, 90 chicks were randomly divided into 3 groups, named 1, 2, and 3, comprising 30 chicks each and bled for pre-vaccination sera. Groups 1 and 2 were vaccinated according to the 2 vaccination schemes mentioned in Table 1. Group 3 was kept as an unvaccinated control.

Sampling Schedule

Ten randomly selected birds from each group having the same maternal antibody titer were taken. Blood samples from these birds were collected on days 1, 5, 14, 21, 34, 42, 54, and 61. Sera were separated and stored at –20 °C until further use.

Challenge with virulent field virus

Twenty birds from each group were isolated and challenged on day 42 with velogenic NDV at a dose rate of 0.1 ml per bird administered subcutaneously. The birds were kept under observation for 19 days for the development of clinical signs of the disease or mortality. Postmortem examination of the dead birds was done, lesions (if any) were recorded, and samples were collected for the re-isolation of the causative agent. Numbers of dead and live birds were recorded. The preventable fraction/protection index to evaluate the efficacy of vaccines was calculated as described by Tizard (10).

Determination of lethal dose (LD_{50}) of Newcastle disease virus (NDV)

To calculate LD_{50}, 25 cockerels (28-days old) were procured and divided into 5 groups, namely A_1, A_2, A_3, A_4, and A_5. Each bird in group A_1 was injected intramuscularly with 0.2 ml of 10^{-2} concentration of NDV. Groups A_2

Table 1. Experimental design.

	Vaccination	
Day of vaccination	Scheme A	Scheme B
5	TAD ND Vac La Sota with 10^9 EID_{50} (E/D)	TAD ND Vac La Sota with 10^9 EID_{50} (E/D)
12	IBD (S/C)	IBD (S/C)
17	HPS (S/C)	HPS (S/C)
21	TAD ND Vac La Sota with 10^9 $EID5_0$ (E/D)	Mukteswar (I/M)

Table 2. Geometric mean titer (GMT) in chickens vaccinated with ND vaccines.

Groups	HI GMT before infection on day:			HI GMT after challenge on day:			
	Day 5	Day 14	Day 21	Day 34	Day 42	Day 54	Day 61
1	22.60	137.20	55.70	168.90	32.00	4.60	7.50
2	22.60	128.00	64.00	68.60	8.00	6.10	4.60
3	22.60	4.30	2.50	1.20	13.00	48.20	128.00

Table 3. Protection against challenge with virulent field strain of NDV.

Groups	Total birds	Live birds	Dead birds	Mortality (%)	Preventable fraction/protection Index
1	20	17	03	15	75
2	20	20	00	00	100
3	20	08	12	60	-

through A_5 were inoculated with 0.2 ml of concentration 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, respectively. Birds in all the groups were observed for mortality up to 10 days post-inoculation. The LD_{50} of the virus was determined as described by Reed and Muench (11).

Experimental Parameters

The following parameters were studied:

Antibody titer against NDV:

Antibody titer against NDV in serum was determined by haemagglutination inhibition (HI) test as described by Allan and Gough (12).

Post-challenge mortality:

Post-challenge mortality in chicks of all groups was recorded. A postmortem examination of the dead birds was performed and lesions (if any) were recorded.

Statistical analysis of results

The level of antibody response was analyzed and compared between groups 1 and 2 by using a 2-sample t-test for equal variance as described by Steel and Torrie (13). Significance level was $P < 0.05$.

Results

The antibody response of the chickens to vaccination schemes and resistance to virulent field virus challenge are given in Tables 2 and 3, respectively. After vaccination HI geometric mean titer (GMT) values were determined and it was observed that on day 14 these values increased significantly in both groups (137.00 in group 1 and 128.00 in group 2).

After the booster vaccination, the HI GMT values were recorded as 168.90 in group 1 and 68.60 in group 2 on day 34. The HI GMT values were statistically analyzed and the difference between them was non-significant ($P < 0.05$). The antibody titers decreased gradually after the challenge (day 42) and they were recorded as 7.50 in group 1 and 4.60 in group 2 on day 61. Birds that survived in the control group showed increasing levels of HI GMT, i.e. 128.00 on day 61.

After the challenge, in group 1 the vaccination scheme gave 75% protection and in group 2 it gave 100% protection. Unvaccinated control birds were highly susceptible to challenge; 12 deaths were recorded. The birds that died post-challenge were examined for any postmortem changes. Usually gross lesions were minimal

in young or old birds although there were mild air sacculitis, conjunctivitis, and tracheitis. The other changes observed were haemorrhagic or necrotic focal lesions, present in the mucosa of the intestine.

Discussion

Despite the widespread use of different types of vaccines, ND continues to be a major threat to the poultry industry. Comparative efficacy of commercially available vaccines has been a challenge for scientists. The present study evaluated 2 vaccination schemes for the commercially available live vaccines against NDV to be used under local conditions. The 2 vaccination schemes used in this study include scheme A, in which La Sota (lentogenic) vaccine was administered via eye drop at day 5 of age followed by a booster vaccination with the same vaccine on day 21 of age in broilers, compared with a booster vaccination with a mesogenic vaccine, i.e. Mukhteswar, by i/m route in scheme B.

A number of researchers have reported that live ND vaccines give better protection and health status than killed vaccines (14,15). The use of live vaccines is preferred for priming the birds as it produces local immunity in the mucosal membrane of the conjunctiva, thus providing immediate protection on subsequent exposure with field virus challenge. Folitse et al. (8) also observed that live vaccines induce local immunity followed by inactivated vaccine, which causes slow release of antibodies. Parry and Aitken (7) also found that live ND vaccines administered by eye drop or orally induce protective mucosal immunity mediated by IgA antibodies. It is speculated that the results obtained in our study indicate that the high antibody titer in group 1 on day 34 is due to the booster vaccination (day 21) with La Sota virus, which replicated quickly in the mucosal membrane of the conjunctiva, inducing local immunity.

It is advisable that priming of the birds should be carried out when the maternal antibody titer drops to the level where it does not interfere with the vaccine. In the current study, the initial vaccination was carried out at the time when the maternal antibody titer (GMT 128) was diminishing (GMT 22.60).

Alexander (3) reported that live lentogenic NDV vaccines produce an antibody titer of 2^4 to 2^6. However, higher HI titers (as high as 2^{11} or more) may be obtained following a vaccination program involving oil-emulsion vaccines. Westbury et al. (16) also observed that La Sota is much more immunogenic than the Hitchner B1 and strain V4. Giambrone (17) conducted an experiment to evaluate a ND vaccination program and reported that NDV HI titers were highest and resistance to challenge is greatest in birds initially vaccinated at day 1 with a live vaccine by coarse spray and then revaccinated at day 14 by the same vaccine by the same route. The increase in antibody titers recorded after the primary vaccination (GMT 137.00 in group 1 and 128.00 in group 2) in the present study is in agreement with these observations.

According to Alexander (3), the actual titers obtained and their relationship to the degree and duration of immunity for any given program are difficult to predict. In the current study antibody titers in group 2 were also unpredictable. The drop in HI titers (GMT 68.60) after the booster vaccination is speculated to be due to the intramuscular injection of the mesogenic strain of NDV (Mukteshwer), which induced a secondary immune response, but the antibodies circulating in the serum, which are detectable by HI test, were low in number. These low-HI detecting antibodies provide immunity and protection to chickens up to 41 days post-vaccination.

A post-challenge decrease in HI antibody titer was observed in birds receiving vaccination as compared to the non-vaccinated control, in which the HI antibody titer was increased. These findings are in line with the findings reported by Tizard (18), which showed that antibody titer was decreased due to neutralization of the virus by circulating antibodies.

On the basis of post-challenge mortality, group 2 showed 0% mortality against challenge with NDV as compared to group 1. This indicates that birds initially vaccinated with La Sota (with 10^9 EID_{50}) and revaccinated with Mukteswar strains had 100% protection, although HI antibodies titers were low. Therefore, it is speculated that, in addition to the protection by HI antibodies, other mechanisms of protection apparently played a part. Lower mortality in La Sota vaccinated birds followed by inactivated NDV was also reported by Mrzel et al. (19). These results are also consistent with the findings reported by Winterfield et al. (20). Khadzhiev et al. (21) also found that, out of 4 vaccination schemes, better production index results were obtained from birds vaccinated with La Sota by the spray method followed by Komarov (mesogenic) strain given intramuscularly.

From the above discussion it can be concluded that a vaccination scheme having La Sota (with 10^9 EID_{50}) as primary vaccine followed by Mukteswar strain vaccines had more protective effects on the host than La Sota (with 10^9 EID_{50}) alone. No significant differences were observed in the HI antibody titer between La Sota (with 10^9 EID_{50}) followed by Mukteshwer and La Sota (with 10^9 EID_{50}) alone vaccines, but a significant difference was found between the protective index of schemes A and B. Therefore, scheme B can be recommended for an effective ND control program under local conditions (Pakistan).

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