

Preparation of Newcastle Disease Vaccine from VG/GA Strain and its Evaluation in Commercial Broiler Chicks

Usman Waheed^{1,2}, Muhammad Siddique², Muhammad Arshad², Muhammad Ali^{3*} and Ali Saeed³

¹Department of Microbiology, College of Veterinary and Animal Sciences, Jhang sub-campus University of Veterinary and Animal Sciences, Lahore, Pakistan

²Department of Veterinary Microbiology, Institute for Microbiology, University of Agriculture, Faisalabad, Pakistan

³Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan, Pakistan

Abstract- Newcastle disease (ND) – an infectious disease of poultry – is the cause of huge economic losses (billions of rupees per annum) to poultry industry of Pakistan. Vaccination of birds is the only way to control this disease. Apathogenic strain of NDV, VG/GA, is in routine use as live vaccine in poultry sector. In present study we prepared an oil emulsified (OE) ND vaccine from VG/GA strain and evaluated it in commercial broilers at farm level. Virus was propagated in 10-days-old chicken embryonated eggs and confirmed by haemagglutination (HA) test with subsequent virus killing with formalin (0.12%). Hydrophile-lipophile balance was maintained at 7.0. Sterility, safety and stability and residual infectivity of the prepared vaccine was checked. The efficacy of the vaccine was evaluated in commercial broiler chicks (n=2200) divided into three groups i.e. imported, experimental and un-inoculated control group at farm level. . The blood samples were collected at day zero of inoculation and then up to 8 weeks post vaccination (PV) with week interval. Indirect Haemagglutination (IHA) test was applied and peak levels of Abs were observed in vaccinated groups till the 2nd week PV which declined gradually for next two weeks with no statistically significant difference among vaccinated birds. On contrast, there was no change in levels of Abs of control group with significant differences compared to vaccinated birds. Birds (randomly selected) from each group were challenged with very virulent field NDV to check protection against challenge, which showed 75% protection in both imported and experimental vaccine inoculated groups. While, uninoculated control group showed only 25% protection. The OE vaccine of ND can be prepared locally with satisfactory results saving foreign exchange.

Key words: Newcastle disease vaccine, VG/GA strain, NDV, broiler chicken, haemagglutination test.

INTRODUCTION

Newcastle disease (ND) - worldwide problem with severe economic implications, affecting chickens, turkeys and other birds - is caused by virulent strains of paramyxovirus type 1 (APMV-1) (Miller *et al.*, 2010b). The virus belongs to family Paramyxoviridae of *Avulavirus* genus and exist in ten serotypes namely APMV-1 to APMV-10 (Miller *et al.*, 2010a). The high pathogenicity of the virus is responsible for the serious economic losses in Pakistan (Jaffery, 1985). In Pakistan, poultry industry is facing billions of rupees of losses due to ND every year. Airborne infection to other birds is possible due to resistance of virus in the environment. In-addition, immune birds become carrier too (Allan *et al.*, 1978).

Four pathotypes of NDV have been described by Spradbrow (1987), which are avirulent (causing no disease), lentogenic (low virulence), mesogenic (moderate virulence) and velogenic (high virulence). On the basis of pathogenicity, in Pakistan, 5% of the field strains were reported as velogenic, 55% as mesogenic and 40% as lentogenic (Azam *et al.*, 1985).

During recent years, injectable oil emulsified (OE) inactivated ND vaccines are popular because of provision of high and more durable levels of immunity as compared to live ND vaccines (Brugh and Siegel, 1978; Eidson *et al.*, 1980). The use of surfactants, oils and emulsion type for inactivated ND vaccines were studied extensively by Cajavec *et al.* (1996) and it was found that a good level of protection against the field strains can be achieved with ultimate reduction of losses to the farmer and industry.

The use of LaSota or B1 live ND vaccines for priming of birds with subsequent inactivated OE vaccine gave good protection when challenged with

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very virulent NDV (VVNDV) strain (Mohammadi *et al.*, 1996). The incidence of VVNDV declined dramatically owing to the introduction of different types of ND vaccines in commercial poultry (Folitse *et al.*, 1998).

The difference of aqueous to oil ratio in preparation of inactivated OE ND vaccine prepared from Mukteswar strain of ND was observed by Mahboob *et al.* (1999) and he found that 1:2 ratio is the best for the purpose and conferred 80% protection when challenged with VVNDV. Presently, in Pakistan all killed vaccines are imported ones with high costs. An inactivated oil emulsion ND vaccine had already been prepared from VG/GA strain of NDV, and had been evaluated experimentally in the Department of Veterinary Microbiology, University of Agriculture Faisalabad. The investigation was initiated to prepare inactivated vaccine from VG/GA strain of NDV and to perform field trials with the aim to check its safety and efficacy in the field conditions.

MATERIALS AND METHODS

Commercial live vaccine of ND having VG/GA strain (Avinew, Rhone Merieux, France) was procured for the production of experimental vaccine. The Imopest ND killed vaccine (Rhone Merieux, France) was purchased for comparative evaluation. Two thousand and two hundred commercial broiler birds present in a commercial poultry farm (Umair Poultry Farm, Chak No. 200/R.B., Faisalabad) were selected for present studies. Five 10 days old chicken embryonated eggs were inoculated per allantoic cavity route with 0.1 ml of reconstituted virus as described by Allan *et al.* (1978). After 72 h post-inoculation, all the embryos were chilled at +4°C for 12 h (Trybala, 1987). The allanto-amniotic fluid (AAF) was harvested as described by Senne (1989).

Allantoic fluid from each egg was tested for haemagglutinating activities by spot HA test in microtitration plates (Titre Tech. ICN, UK). Inactivation of the harvested NDV was carried out by formalin (Merck: 37.5%) at a final concentration of 0.12% (Cardona *et al.*, 1987; Reddy and Srinivasan, 1991) with later on incubation at 37°C for 24 h (Chand, 1998). Residual infectivity of the

virus was checked in embryonated eggs (Mahboob *et al.*, 1999). Oil and aqueous phase adjuvant (Span 80 and Tween 80) were used with 10% of surfactants for stable emulsion type. The final ratio of AAF to the adjuvant was 1:4. Physical properties of experimental and imported vaccines were noted (Griffin, 1979). Stability of experimental and imported vaccines was evaluated by placing the vaccines at different temperatures (4°C, room temperature and 37°C). Here the stability means the time required for the separation of oil phase from aqueous phase in the vaccine vial. Sterility of the AAF was checked in thioglycolate medium and pleuro-pneumonia like organism (PPLo) broth.

All birds were primed with live NDV vaccine at 7 days of age with live LaSota ND vaccine. For evaluation of vaccine at farm level under same conditions, three groups *i.e.* experimental and imported vaccine inoculated (n=1000 each) and unvaccinated control group (n=200) was made. Blood from wing vein of 8 randomly selected birds was collected in BD vacutaniers at day zero of inoculation and then with week interval up to six weeks. The serum was isolated by separating the blood clot after centrifugation at 4000 rpm in clinical centrifuge and kept at -20°C till processing for detection of Abs titers. Antibodies titers of serum samples were determined by haemagglutination Inhibition (HI) test as described by World organization for Animal Health (2012). Geometric mean titers (GMT) and cumulative mean titers (CMT) of Abs in the sera of each group were determined as narrated by Mahboob *et al.* (1999).

At six weeks postboosting, the birds of each group were challenged by injecting the VVNDV, with 64 HA titre at 0.1 ml/per bird, *i.m.* in the thigh region. The birds were monitored for 7 days and morbidity and mortality pattern in each group was recorded. The results were analyzed statistically (Steel and Torrie, 1980).

RESULTS

The present study was planned to prepare an OE formalized ND vaccine from VG/GA (apathogenic) strain and to evaluate this in field conditions. Five 10-day-old chicken embryonated eggs were inoculated with reconstituted VG/GA

strain of NDV. The harvested AAF showed HA titer of 512. The harvested AAF again inoculated into other set of twenty five, 10-days-old embryonated eggs. Spot HA positive AAF were pooled and the HA titer of pooled AAF was 2048.

The colour of both the vaccines, *i.e.* experimental and imported vaccine was milky white. Experimental vaccine was relatively more viscous (flow time 3.0 seconds) than imported vaccine (2.5 seconds). The emulsion type of both the vaccines was water-in-oil (W/O). The vaccines were stable for 20 weeks at 37°C and room temperature. However, at 4°C, experimental vaccine was not stable for more than 3 days, whereas the imported vaccine remained stable for 7 days.

For safety test, broiler and golden chicks were inoculated with experimental vaccine intramuscularly and monitored for 7 days. The birds remained quite active with normal feeding and water intake. There was no change in colour and consistency of feces. At the injection site, a small granulomatous lesion was observed by fourth day of inoculation, otherwise there was no tissue reaction. For sterility testing, no growth was observed on blood agar, thioglycolate and PPLO broth even after 14 days of incubation. The HI titres against ND in all three groups on day zero were ranging from 16-128 and GMT showed non-significant results. At 2nd week postboosting, significant differences in GMT were recorded in vaccinated and unvaccinated control group, while the difference of GMT values in experimental and imported vaccine groups were non-significant. The same pattern of GMT was observed in the sera of birds collected after 3rd week of postboosting. No significant difference was observed among the vaccinated groups but significant variation was found between vaccinated and control group of birds. The same pattern of HI antibodies was observed after 4, 5 and 6 weeks postboosting. The GMT and cumulative mean values of all the groups are summarized in Table I.

The statistical analysis of the HI titres at second week postboosting showed significant difference among the three groups. This indicated that there was significant increase in the HI antibodies against ND two weeks after boosting among vaccinated and unvaccinated control group. The peak of HI titres was observed at two weeks

postboosting. The statistical analysis of the HI titres at third week postboosting indicated significant difference among the antibodies level of the birds against ND in vaccinated groups as compared to the unvaccinated control group three weeks postboosting. The statistical analysis of the titres of HI at four weeks postboosting revealed significant difference against ND in the groups which were vaccinated in contrast to the unvaccinated control group. The statistical analysis of the HI titres of three groups at five weeks postboosting, showed significant difference of antibodies against ND. The statistical analysis of HI titres against ND at six weeks postboosting indicated significant difference of antibodies against ND among the vaccinated and non-vaccinated birds.

CMT were calculated on the basis of GMT of the three groups at various intervals postboosting. The CMT values were 1036.98, 888.81 and 52.25 for experimental, imported vaccinated and control groups, respectively (Table I). The protection against challenge exposure was recorded as 75, 75 and 25% in experimental, imported vaccinated and control groups, respectively.

DISCUSSION

The live and killed vaccines have their own merits and demerits. Live vaccines are relatively inexpensive and lend themselves to mass application. Local immunity is being stimulated by vaccination with live viruses and protection occurs very soon after application. Vaccinal viruses spread easily to all the birds in the flock. Among disadvantages of live attenuated vaccines, the most important are the vaccine may produce immunosuppressive effects, especially in young ones, mixing of extraneous agents which may induce disease in recipients and keeping in view of maternal antibodies in the birds before vaccination (Quinn *et al.*, 2002). Because of this, it is extremely important to use mild viruses for primary vaccination and, as a result, multiple applications for vaccines are usually needed. Spread of live viruses could cause severe disease problems. Live vaccines have to be stored around 4°C. Live virus vaccines may have very adverse affects on the maternal immunity if the birds that have been

Table I.- Geometric mean titre values of each group of birds.

Group	Geometric mean titers (weeks post-boosting)						Cumulative mean
	0*	2 nd	3 rd	4 th	5 th	6 th	
Experimental	55.7	1097.5	1024.0	955.4	1176.3	1024.0	888.81
Imported	52.0	1260.7	1176.3	1024.0	1448.2	1260.7	1036.9
Control	48.5	52.0	48.5	48.5	64.0	52.0	52.25

* Serum sample collected just before vaccination.

derived from vaccinated flock, so live vaccines cannot be used in day-old chicks (World Organization for Animal Health, 2012; Box *et al.*, 1976).

NDV of low virulence induces similar immune responses without causing severe disease. This is the basis of vaccination (FAO, 2012). Killed vaccines have the advantages like, the vaccines are easy to store at room temperature and there is no need to keep at ambient temperature during transportation. Very few adverse reactions due to vaccination can be anticipated in inoculated birds. Inactivated OE vaccines are not as adversely affected by maternal immunity as live vaccines and thus can be used in day-old chicks (Box *et al.*, 1976). Killed vaccines evoke high level of circulating antibody response for long duration (Eidson *et al.*, 1980; Ernawati and Ibrahim, 1984).

In Pakistan, presently, OE ND vaccines are being imported and it consumes a reasonable foreign exchange. Various strains of NDV are being used for the production of killed vaccines. Mahboob *et al.* (1999) prepared killed OE vaccine from LaSota strain of NDV. Rehman *et al.* (2002) prepared vaccine from VG/GA strain of NDV and conducted studies on the efficacy of the vaccines under experimental conditions. Chand (1998) conducted a study on the preparation of OE vaccine from mesogenic strain of NDV. The vaccines gave high serum Abs level and good protection against challenge in both the experiments under controlled environmental conditions. The present study was the continuation of previous work (Rehman *et al.*, 2002) to find out the effectiveness of vaccine under field conditions. Rehman *et al.* (2002) studied the preparation and evaluation of OE inactivated ND vaccine under experimental/controlled conditions. The present study dealt with the preparation and

evaluation of OE inactivated ND vaccine under field conditions.

For the production of killed vaccines, formalin (0.12%) was used for inactivation of NDV. Although other substances were also in use like β -propiolactone (Kolbel, 1985). Formalin was preferred as inactivating agent for vaccine production because formalin has a good viricidal as well as bactericidal effect (Cardona *et al.*, 1987; Reddy and Srinivasan, 1991). Tween-80 and Span-80 were used as surfactants at a ratio of 10% to the mineral oil. According to Cardona *et al.* (1987), the vaccine which had 10% of surfactants to oil phase (mineral oil) gave high level of antibodies and protection against challenge. Mahboob *et al.* (1999) and Rehman *et al.* (2002) also used the same surfactants at the same ratio (10%) to the oil phase. Our findings about the physical properties of the experimental and imported vaccines were in line with the findings of Stone *et al.* (1978) and Cardona *et al.* (1987). Imported vaccine was stable for longer period of time at 4°C (7 days) as compared to experimental vaccine (3 days). The reason for this may be the difference of aqueous to oil ratio, the type and number of surfactants used, the concentration of the surfactants used and difference of HLB. Generally physical properties of OE vaccines are very much affected by the factor such as type of emulsifiers used, ratio of the aqueous phase to the oil phase and procedures of emulsification (Stone *et al.*, 1978). Newcastle disease killed vaccine gave better results if the chicks were primed with live ND vaccine, 7-14 days before vaccination (Lin *et al.*, 1990; Mahboob *et al.*, 1999; Rehman *et al.*, 2002). In the present trials, LaSota live vaccine was used 7 days before vaccination for priming (7 day of age). However, various other live NDV strains may be used for

priming the birds. In Pakistan, most of the poultry raising areas showed excellent results without priming. This may be due to the factor that various NDV strains are well spread everywhere and there is natural priming.

The trend of HI antibodies increase in present study was; the peak of titres was achieved two weeks after boosting, which gradually decreased for the next two weeks. At five weeks postboosting there was a sudden increase in the titres of all the three groups. The reason for this sudden increase in titres might be that there was an outbreak of ND in the adjacent open poultry farm. At six weeks postboosting again gradual decrease in HI titres was observed. The same had been reported by Cajavec *et al.* (1996) and Tan-Jian *et al.* (1996).

The protection against challenge test was conducted at 6 weeks postboosting (VanEck, 1990; Awad *et al.*, 1993). In the present studies, the protection was 75% against the challenge in both experimental and imported vaccines. While the unvaccinated control showed only 25% protection against the challenge and similar findings were found by other scientists (Hassan *et al.*, 1992).

There would be high level of serum HI antibodies against ND after two weeks post-boosting with killed OE vaccine (Awad *et al.*, 1993; Chen *et al.*, 1993; Cajavec *et al.*, 1996). So, the protection against challenge test could be conducted after 14 days of boosting. The results which would be obtained at that point of time would be reliable and true representative of the studies. The cost of the experimental vaccine to the imported vaccine was compared, which was half of the cost to the imported vaccine. This huge difference of the cost of vaccine production would lead to local manufacturing of OE inactivated ND vaccine. This would protect the birds from ND and ultimately reduce the high economic losses due to the import of ND vaccines in Pakistan.

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