



## Research Article

# Post Vaccinal Antibody Profile against Different Vaccines of Newcastle Disease in Backyard Poultry

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### ABSTRACT

Three different types of Newcastle disease (ND) vaccines were compared in the study on the basis of their post vaccinal antibody titres in backyard poultry. A total of one hundred domestic poultry birds were selected and divided into five equal groups (A to E). All the groups except Group E (absolute control) were primed with ND La Sota vaccine at 7<sup>th</sup> day of age. At day 21 groups A, B, C were boosted with ND La Sota, Mukteswar (R2B strain) and ND killed vaccine (oil based) respectively, while group D was kept as booster control. Antibody titres were compared by using the Haemagglutination Inhibition (HI) Test. Six weeks cumulative mean titres (CMT) recorded in groups A, B, C, D, and E were 36.22, 51.22, 57.97, 15.23 and 5 respectively. Statistical analysis showed that the CMT of groups vaccinated with the booster dose of Mukteswar (R2B strain) and oil based vaccines were significantly higher ( $P < 0.05$ ) from the group vaccinated with the live La Sota vaccine. The results indicated that booster vaccination with Mukteswar (R2B strain) or oil based vaccine should be used rather La Sota.

**Key words:** Newcastle disease, Backyard poultry, Haemagglutination inhibition, LaSota, Mukteswar

### INTRODUCTION

Newcastle Disease Virus (NDV) belongs to the Avian Paramyxovirus Serotype 1 (APMV-1) (Alexander *et al.*, 2003) and it is tentatively placed in the genus *Rubulavirus* (Van Regenmortel *et al.*, 2000) belonging to the subfamily *Paramyxovirinae*, family *Paramyxoviridae*, order *Mononegavirales* (Snoeck *et al.*, 2013). Virion is round and 100-500 nm in diameter. It is an enveloped RNA virus presentation helical capsid symmetry with non-segmented single strand of negative polarity (Samson, 1988). The genome codes for 6 proteins including the Haemagglutinin (H), Neuraminidase (N), Matrix (M) protein, Nucleo-Protein (NP) and Fusion (F) protein (Guan *et al.*, 2010). There are four subtypes of this virus based on pathogenicity and postmortem lesions in chickens: 1) lentogenic; 2) mesogenic; 3) velogenic neurotropic; 4) and velogenic viscerotropic (Michelle, 2004).

Poultry eggs and meat are valuable source of protein in the era of protein insufficiency in Pakistan. The

products from rural poultry are always ranked higher by the consumer due to delicious taste. Poultry industry in Pakistan is the back bone of commercial as well as rural economy. Almost every family in rural areas and every fifth family in urban areas is associated with poultry production in one way or the other. It contributes significantly to Nation's GDP. There are about 931.30 million poultry birds in Pakistan, among, which rural poultry is about 83.32 millions. It plays a vital role in the village economy with the contribution of up to 4018 million eggs and 112.99 thousand tons of the poultry meat (Pakistan Economic Survey, 2014-15).

Newcastle disease (ND) is one of the most important cause of mortality in chickens (Bulbule *et al.*, 2015). It is a major constraint to village poultry production throughout developing countries, frequently causing mortality rates of 75-100% in unvaccinated flocks (Spradbrow, 1992). Inactivated vaccines give very good immunity without vaccinal reactions and have been widely used. Live vaccines are easy to apply and relatively inexpensive, and give moderately good

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immunity (Bell, 1995). In countries like Viet Nam, Nigeria and Switzerland many studies were conducted on ND in backyard poultry but currently no data is available regarding post-vaccinal antibody profile of ND in backyard poultry in Pakistan. Therefore the present investigation was conducted to evaluate the efficiency of different commercially available vaccines of ND in backyard poultry.

## MATERIALS AND METHODS

### Vaccines

Three different vaccines of ND, procured from local market, were evaluated on the basis of post vaccinal antibody titers in the backyard poultry chickens:

- 1) Live La Sota vaccine
- 2) Live Mesogenic Mukteswar (R<sub>2</sub>B) strain vaccine
- 3) Inactivated oil-based ND vaccine

### Fertile eggs

One hundred and fifty fertile (n= 150) eggs of backyard poultry were collected from the different rural areas of Faisalabad and placed in the egg incubator in the Institute of Microbiology, University of Agriculture Faisalabad. After proper disinfection at 99.5°F, 55% relative humidity (70% in last three days of incubation) eggs were turned regularly to get the day old chicks.

### Experimental design

A total of 100 day old chicks were kept in experimental poultry shed and were divided into five groups (A to E), each group having 20 chicks (Table 1). Groups A to D were vaccinated with La Sota strain vaccine on 7<sup>th</sup> day by eye drops and group E was kept absolute control. At the age of 21 days, the chicks of group A were vaccinated with La Sota strain vaccine via eye drops, group B with Mukteswar strain vaccine S/C and group C with inactivated oil adjuvanted vaccine S/C. The chicks of group D were kept as booster control and that of group E were kept as absolute control.

### Collection of Blood Samples

Blood samples 3-5 ml (without anticoagulant) were collected from at least three chicks randomly from each group on day 0, 7, 14, 21, 28, 35 and 42 post vaccination. Blood was transferred to sterile test tubes to stand in slant position and stored in refrigerator overnight to collect serum. The serum was collected after centrifugation at 2000 rpm for 10 minutes and was stored at -20°C for further use in HI test as described by Mahmood *et al.*, (2004).

### Statistical analysis

Geometric Mean Titer (GMT) was calculated by using the serum titers for each experimental group at weekly intervals. Cumulative Mean Titer (CMT) was calculated by using the serum titer of each experimental group in all the weeks. Statistical differences among GMTs and CMTs of different groups within each experiment were estimated using the analysis of variance (ANOVA) and means were compared by applying Duncan's multiple range (DMR) test (Duncan, 1955; Steel and Torrie, 1980).

## RESULTS

There was a non significant ( $P>0.05$ ) difference in humoral immune response at day 0 of age among all the groups. The GMT ranged from 4.76 to 9.51 (Table II). At 7<sup>th</sup> and 14<sup>th</sup> day post vaccination, the GMT of vaccinated groups had significant difference as compared to unvaccinated group F at  $P<0.05$ . These results revealed that antibody titers of vaccinated groups increased as compared to unvaccinated group by using the La Sota vaccine, which is usually used as primary vaccine in backyard and commercial poultry.

At the age of 21 and 28 days, there was sharp increase in titres of group A to C. This increase in titres was due to the effect of priming at the age of day 7. La Sota vaccine (Merial, France) gave better results as compared to local strain. The results of group D showed the decline in titer produced by the live vaccine on primary vaccination, but still significantly differed from the absolute control group E at  $P<0.05$ .

At 35<sup>th</sup> day post vaccination, GMT of both groups B and C had significant difference as compared to other groups but GMT of group C had no significant difference ( $P>0.05$ ) as compared to group B. The titers produced by the live La Sota vaccine started declining (group A) but the titers produced by live Mukteswar strain vaccine and oil-based vaccine were again on higher side. At 42<sup>nd</sup> day of post vaccination, GMT of group C had significant difference ( $P<0.05$ ) as compared to group B and the titers produced by all the other groups continued declining.

Cumulative Mean Titer (CMT) of group A to E was recorded as 36.22, 51.22, 57.97, 15.23 and 5. The group C showed the best CMT (57.97) and group E showed the least CMT (5). Statistical analysis showed that the CMT of group B and C significantly differed from CMT of group A, which differed from group D and that differed from group E.

## DISCUSSION

Killed and live vaccines have some advantages and disadvantages. Live vaccines have advantage that they tend to be cheaper and it is much easier to apply them on large scale with minimum labor required. Live vaccines have also got the capability to stimulate the mucosal immunity and can offer rapid protection to the birds following application. Following live vaccine, spread of the virus occurs rapidly among the whole flock due to multiplication of the virus. But their main disadvantage is that they tend to suppress the immune system of the very young birds and must be used in accordance with the level of maternal antibody levels. Furthermore these need to be stored at 4°C (Quinn *et al.*, 2002). On the other hand, efficacy of the killed vaccines is not interfered by the temperature and there is no need to be stored at 4°C. Killed vaccines do not tend to interfere with the maternal immunity although the development of protective immunity is slower with the use of killed vaccines. However, they induce the protective level of immunity for a longer duration (Eidson *et al.*, 1980; Ernavati and Ibrahim, 1984).

In Pakistan, currently, most of the ND vaccines are imported and this leads to huge burden on national economy.

**Table 1:** Experimental Model for the trial

Groups	Birds	Vaccine day 7	Route	Vaccine day 21	Route
A	20			La Sota	Eye drops
B	20	La Sota	Eye Drops	Mukteswar	S/C
C	20			Oil-based	S/C
D	20			Booster Control	
E	20			Absolute Control	

**Table 2:** Haemagglutination Inhibition (HI) Titers of different groups (weekly interval)

Groups/ Weeks	A	B	C	D	E
1	06.73 <sup>a</sup>	04.76 <sup>a</sup>	06.73 <sup>a</sup>	06.73 <sup>a</sup>	09.51 <sup>a</sup>
2	13.45 <sup>a</sup>	16.00 <sup>a</sup>	13.45 <sup>a</sup>	19.03 <sup>a</sup>	08.00 <sup>a</sup>
3	26.91 <sup>a</sup>	26.91 <sup>a</sup>	32.00 <sup>a</sup>	38.05 <sup>a</sup>	05.66 <sup>b</sup>
4	64.00 <sup>a</sup>	76.11 <sup>a</sup>	53.82 <sup>b</sup>	26.91 <sup>c</sup>	04.76 <sup>d</sup>
5	128.00 <sup>ab</sup>	152.76 <sup>ab</sup>	181.02 <sup>a</sup>	19.03 <sup>c</sup>	04.00 <sup>d</sup>
6	76.11 <sup>b</sup>	215.22 <sup>a</sup>	256.00 <sup>a</sup>	09.51 <sup>c</sup>	03.36 <sup>d</sup>
7	53.82 <sup>b</sup>	181.02 <sup>ab</sup>	304.44 <sup>a</sup>	08.00 <sup>c</sup>	02.83 <sup>d</sup>
CMT	36.22 <sup>b</sup>	51.22 <sup>a</sup>	57.97 <sup>a</sup>	15.23 <sup>c</sup>	05.00 <sup>d</sup>

Means sharing the same superscripts do not differ at ( $P < 0.05$ ); Group A: ND La Sota; Group B: Mukteswar strain; Group C: Oil-based; Group D: Booster control; Group E: Absolute controls

However many local workers are doing efforts in the development of ND vaccines with promising results. Arshad *et al.*, (2005) prepared and studied the immunological parameters of ND vaccine of mesogenic strain (oil emulsified). Mahbbob *et al.* (1999) prepared the OE-ND vaccine from the LaSota strain. Rehman *et al.* (2002) experimentally prepared the ND vaccine from the VG/GA strain of NDV. All of these OE vaccines resulted in generation of higher antibody titers and good protection against clinical disease. In these, 0.12 % formalin was used for the inactivation of the virus. Formalin possess good antibacterial as well as antiviral effects. Span 80 and Tween 80 were used as surfactants (10% of the mineral oil used) as prescribed by the Cardona *et al.* (1987).

The present study showed that the booster vaccination with Mukteswar strain of vaccine gave better results as compared with when the booster vaccination is again with La Sota vaccine against ND. The similar results were reported by Ahmad *et al.* (2006). A number of other researchers also reported that killed vaccines of ND gave better post vaccinal antibody titers when the chicks were given priming dose with live vaccine 7-14 days before (Lin *et al.*, 1990; Mahboob *et al.*, 1999; Rehman *et al.*, 2002, Kafi *et al.*, 2003). But these results were not in agreement with the Rehmani (1996) who stated that using Mesogenic strain of ND (Mukteswar strain) resulted serologically inferior humoral immune response as compared with that of Lasota strain of NDV. In the current study, oil-based inactivated vaccine produced antibody titers, which were not only higher than live vaccines but they remained fairly consistent for longer period. These results agreed with the findings of Alexander *et al.* (2003) who reported that HI is more accurate for assessment of field protection than test results from enzyme-linked immunosorbent assay (ELISA) kits. The inactivated oil adjuvanted ND vaccines gave good results when the birds were primed with live vaccines as compared with when vaccination was done by live vaccines which has also been reported by Ahlers *et al.* (1999). These findings are in concomitant agreement with the present study that the oil-based vaccine gave better and persistent results than other live vaccines when primed with live vaccine (La Sota) against ND although

the titers produced by the live vaccines (La Sota and Mukteswar) were good and they reached to peak quickly but they dropped quickly also when compared with oil-based vaccine.

### Conclusion

Results of present study concluded that primary vaccination should be with La Sota strain against ND but this vaccination is not sufficient alone. Farmer should at least vaccinate the birds again after two weeks. For better and persistent results it is recommended to use the oil-based vaccine as booster vaccination.

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