# COMPARATIVE EFFICACY OF FIVE DIFFERENT BRANDS OF COMMERCIAL NEWCASTLE DISEASE LASOTA VIRUS VACCINES IN BROILERS

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#### ABSTRACT

Five commercial LaSota strain Newcastle disease (ND) vaccines namely A, B, C, D and E were evaluated for their potency, efficacy, thermostability and influence on productivity in broilers. A 3-log<sub>10</sub> difference of EID<sub>50</sub> and two-to-eight fold difference of HA activity was found among the various vaccines tested. One hundred and fifty day-old broiler chicks were divided into six equal groups tagged as I, II, III, IV, V and VI. The birds in groups I, II, III, IV and V were actively immunized against ND on days 7 (eye drop method) and 21 (drinking water) using vaccines A, B, C, D and E, respectively. The birds in group VI served as unvaccinated control. The serum HI antibody response to five vaccines was determined 7, 14, 21 and 28 days post-vaccination. Fifteen birds from each group including unvaccinated control were challenged at day 35 with local virulent ND field isolate. The HI serum antibody profile and post-challenge mortality pattern revealed a dose-response relation between the virus content, humoral antibody response and clinical protection. To compare the heat stability, the vaccines were incubated at 4, 25 and 40°C for a period of 24 hours. There was no remarkable reduction in HA titer, however slight dips (less than 2 logarithmic units) in EID<sub>50</sub> values were found in all the vaccines. All the vaccines caused significant suppression in weight gain, leading to a poor performance in terms of feed conversion ratio (FCR) and European Efficiency Factor (EEF).

Key words: Newcastle disease, LaSota virus, vaccine, potency, efficacy, thermostability, broilers.

#### INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease, which affects almost all species of domestic and wild birds. This disease is caused by a virus of genus Avulovirus, subfamily Paramyxovirinae, family Paramyxoviridae (Al-Garib et al., 2003). The disease was first recognized in Indonesia and England in 1926 (Doyle, 1927) and ND viruses are now found worldwide. In Pakistan, ND outbreaks are still common, despite the use of massive immunization against ND in various kinds of commercial poultry such as layers, broilers and breeding flocks. The signs of ND can range from no symptoms or mild air sacculitis to severe nervous and/or visceral involvement, leading to paralysis and death of the infected chickens.

In Pakistan, the broiler chickens are routinely vaccinated against ND using various routes of vaccination such as drinking water, intraocular, intranasal and aerosol spray. The age of the chicks at vaccination and the level of maternally derived antibody greatly influence immune response of broiler chickens to the vaccinal antigen. There has been a concern in the broiler industry that ND vaccines being used in Pakistan may not be provoking desired level of antibody response and as such the required protection. Therefore, the purpose of this investigation was to compare five major commercial ND (LaSota) vaccines being marketed in Pakistan for use in broiler chickens with respect to potency, efficacy, thermostability and influence on productivity.

#### MATERIALS AND METHODS

## Vaccines

The representative vials of five major brands of the live NDV LaSota strain vaccines namely A, B, C, D and E were procured from the local market. The vaccines were evaluated for their potency, efficacy, thermostability and influence on productivity of broilers.

## **Potency testing**

The vaccines were assayed for 50% infectivity (EID<sub>50</sub>) and haemagglutinating activity (HA) using eggs from desi (local) hens. The EID<sub>50</sub> of the vaccines was determined using the technique of Reed and Muench (1938). Similarly, HA titer was determined by treating 50 ul of virus suspension in each vaccine vial with 50 ul of 0.5% chicken RBCs suspended in normal saline and by incubating the test material at room temperature for 30 minutes according to the procedure suggested by Allan and Gough (1974).

## **Efficacy testing**

One hundred and fifty day-old broiler chicks were divided into six equal groups (n=25) tagged as I, II, III, IV, V and VI and were managed separately to prevent cross contamination or horizontal spread of vaccinal virus. The birds in groups I, II, III, IV and V were actively immunized against ND on day 7 (eye drop method) and 21 (drinking water) using vaccines A, B,

C, D and E, respectively. The birds in group VI served as unvaccinated controls. The serum HI antibody response to different vaccines was determined 7, 14, 21 and 28 days post-vaccination. Fifteen birds from each group including unvaccinated control were challenged on day 35 with velogenic NDV field isolate.

## Thermostability and productivity

To compare the heat stability, five vaccines were incubated at 4, 25 and  $40^{\circ}$ C for a period of 24 hours. The trends in decline in EID<sub>50</sub> and HA titers were monitored.

The average weight gain and feed consumption were measured for birds of each group on weekly basis. The performance of the birds was evaluated on the basis of feed conversion ratio (FCR) and European Efficiency Factor (EEF). The FCR and EEF were calculated using the following formulas:

FCR= feed consumed (g) / weight gain (g)

EEF = (Livability (%) x live weight (kg)) / (age (days) x feed conversion ratio) x 100,

Where:

Livability = 100 - (% dead + % rejected).

## Statistical analysis

The data collected through the study from various treatment groups were compared by one-way analysis of variance. Differences among treatment means were compared using Least Significant Difference (LSD) test at 5% probability level (Steel and Torrie, 1980).

### RESULTS AND DISCUSSION

A 3-log<sub>10</sub> difference of EID<sub>50</sub> and two-to-eight fold difference of HA activity was found among five vaccines (Table 1). The minimum virus level for live virus LaSota strain ND vaccines is 10<sup>6.5</sup>/dose (Hofacre, 1986). All but one (vaccine E) of the commercial vaccines titered for this study was above this level. Hofacre (1986) compared the potential of high and low titered commercial ND vaccines and recorded 2-log<sub>10</sub> difference of virus titer elicited by the vaccines.

All the vaccine brands indicated similar trends in antibody production. In the inoculated groups, however, the HI titer differed non-significantly among the vaccinated groups except for the vaccine E that provoked relatively weaker primary and secondary immune responses in terms of HI antibody production (Table 2).

Table 1: Comparative EID<sub>50</sub> and HA activities of five commercial live ND (LaSota strain) vaccines

Vaccine source	EID <sub>50</sub>	HA titer	
A	$10^{7.78}$	512	
В	$10^{8.80}$	1024	
C	$10^{8.32}$	512	
D	$10^{7.31}$	512	
E	$10^{4.83}$	128	

Table 2: Comparison of NDV geometric mean HI titers (log<sub>2</sub>) of the broiler chickens primed on day 7 and boosted at day 21 with live NDV LaSota strain vaccines

Vaccine	HI titer (log 2) at days post-vaccination				
source	1	7	14	21	28
A	2.25	2.75	3.25	5.13	5.25
В	1.25	3.75	4.00	5.75	5.63
C	2.00	3.00	3.50	4.80	5.00
D	1.50	3.00	3.20	5.38	5.38
E	1.75	2.00	2.25	3.00	3.25

Following challenge of vaccinated birds in various groups, protection levels of 22, 7, 13, 20 and 40 percent were recorded for vaccines A, B, C, D and E, respectively. All the unvaccinated control chickens challenged on the same day succumbed to infection indicating 100 percent mortality (Table 3). The challenge-protection study revealed that the degree of protection conferred by the vaccines could be related to serum HI antibody profile of chickens.

Table 3: Post-challenge mortality in chicks receiving live NDV LaSota strain vaccine from various sources

Vaccine source	Mortality
A	3/15(20%)
В	1/15(7%)
C	2/15(13%)
D	3/15(20%)
E	6/15(40%)
control	15/15(100%)

A dose-response relationship has been reported among the virus content, serological response and clinical protection (Spradbrow *et al.*, 1988) in the host chicks. Brugh and Siegel (1978) determined the effect of virus concentration on serum antibody levels and concluded that the virus concentration had significant effects on immunogensity of the vaccines. Stone (1985) presented HA activity of oil-emersion ND virus vaccines as a prediction of efficacy. He related HA activity of the vaccines to degree of protection conferred against velogenic viscerotropic (VV) ND. Maas (2003) also investigated correlation of haemagglutinin-neuraminidase and fusion protein content of killed ND vaccines with haemagglutination-inhibition (HI) antibody response in the chickens.

During the present study the comparative heat stability of various vaccine brands was also evaluated by treating the vaccine vials at 4, 25 and  $40^{\circ}$ C for 24 hours and the effects of temperature on the HA titers and EID<sub>50</sub> of virus were recorded. This study did not reveal any measurable reduction in the HA titers. However, the infectivity potential of virus decreased by less than 2 logarithmic units depending upon storage temperature (Table 4). The HA activity was more thermostable than the virus infectivity.

Table 4: Effect of temperature treatment on the infectivity and haemagglutination activity of the NDV vaccines

Vaccine	Temperature					
•	4 <sup>0</sup> C		25 <sup>0</sup>	C	40°	C
•	EID <sub>50</sub>	HA	EID <sub>50</sub>	HA	EID <sub>50</sub>	HA
A	$10^{7.78}$	512	$10^{7.41}$	512	$10^{6.83}$	512
В	$10^{8.80}$	1024	$10^{8.36}$	1024	$10^{7.76}$	1024
C	$10^{8.32}$	512	$10^{7.94}$	512	$10^{7.41}$	512
D	$10^{7.31}$	512	$10^{6.89}$	512	$10^{6.27}$	512
E	$10^{4.83}$	128	$10^{4.36}$	128	$10^{3.89}$	128

Abdu *et al.* (1998) also studied the effects of storage conditions and temperature on efficacy of ND virus LaSota vaccine. The persistence of immunogenicity by incubation at room temperature for variable storage duration reveals the viability of the vaccines. Heath *et al.* (1991) studied the thermostability of V<sub>4</sub> strain of NDV vaccine. The commercial vaccine in freeze-dried form remained stable upto 3 months at 18-22°C. Tu *et al.* (1998) showed that the freeze-dried ND vaccines lost about 1 log of infectivity upon its storage for 24 days at 30-35°C.

Significant differences in the average weight gain were observed amongst the vaccinated and unvaccinated groups of chickens. The vaccinated birds were found less efficient in converting feed than the unvaccinated birds and in all ND LaSota vaccinated groups weight gain was suppressed (Tables 5 and 6).

Table 5: Feed efficiency performance of chicks at week 5 (mean+ SE)

Vaccine	Feed consumed	Weight gain (grams/bird)	FCR
	(grams/bird)	<b>Q</b>	
A	2443 ±	1361 ±	1.795 ±
	0.31 <sup>a</sup>	1.74 <sup>b</sup>	$0.87^{a}$
В	$2457 \pm$	1382 ±	$1.777 \pm$
	1.73 <sup>a</sup>	$1.72^{b}$	$1.02^{a}$
C	$2452 \pm$	$1367 \pm$	$1.794 \pm$
	$1.72^{a}$	1.73 <sup>b</sup>	$0.96^{a}$
D	$2460~\pm$	1358 ±	$1.811 \pm$
	1.73 <sup>a</sup>	1.15 <sup>b</sup>	$0.112^{a}$
E	$2430  \pm$	1371 ±	$1.772 \pm$
	2.31 <sup>a</sup>	$2.93^{\rm b}$	$0.74^{a}$
Control	$2429 \pm$	$1438 \pm$	$1.759 \pm$
	$2.89^{a}$	2.88 <sup>a</sup>	$0.98^{a}$

Different superscripts on means show significant difference (P<0.05).

Alexander *et al.* (2004) also described the suppressive effect of NDV LaSota strain on weight gain of chickens. The heavy breeds showed more adverse reaction to lentogenic ND viruses. Saif and Nestor (2002) found a positive correlation between increase in the body weight and the increase in mortality following

vaccination with the live LaSota vaccine. Westbury *et al.* (1984) compared the residual virulence of Newcastle disease vaccines strains V4, Hitchner B1 and LaSota in terms of weight gain and number of sneezes. Tu *et al.* (1998) also determined the adverse effect of LaSota vaccines in comparison with ND virus isolates, a thermostable Newcastle disease virus vaccine in experimental and village chickens.

Table 6: Effect of commercial LaSota strain Newcastle disease vaccines on European Efficiency Factor (EEF) in broiler chicks

Vaccine	Liveablity (%)	Live weight (Kg)	Age ( days)	FCR	EEF*
A	80	1527	42	1.795	522
В	93	1588	42	1.777	625
C	87	1563	42	1.794	581
D	80	1534	42	1.811	529
E	60	1471	42	1.772	372
Control	97	1623	42	1.759	596

\*The minimum acceptable value of EEF is 260; the bigger the value the better the performance.

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