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ANTIBODY PROFILE AGAINST NEWCASTLE DISEASE VIRUS IN THE VACCINATED AND UNVACCINATED BIRDS

AQEEL AHMAD*, NOUREEN SAEED, TANVEER ABBAS, MOHAMMAD AHMAD, FASIHUDDIN A. ANSARI, SYED MOHAMMAD KHALID AND LOA'Y I. LUBBAD
Department of Microbiology, University of Karachi, Karachi-75270

ABSTRACT

Poultry, the second largest productive sector in Pakistan, is fast decaying. One of the major losses to this sector is due to outbreaks of different diseases. One of the common diseases in the poultry is Newcastle disease. The best way to decrease incidence of such outbreaks is by providing protection by means of proper vaccination. The effectiveness of the NDV vaccines, available in the market, and their vaccination procedures was studies by immunising birds in different age groups and with various doses, and anti-NDV antibody titre in each bird was measured by ELISA. Then all the birds were challenged with the virulent strain of NDV and survival of the chicks was noted.

Protective levels of antibodies were found in chicken sera up to the age of three weeks due to maternally acquired antibodies. NDV Vaccines, available in market, was found to be effective in developing protective immunity by enhancing the immune response. However the most suitable time for vaccination was found to be the one-week age of chicks.

INTRODUCTION

A large population of Pakistan is engaged in poultry farming. Unfortunately they are confronted with worst ever crisis, which is probably unprecedented in its history. The dramatic cut in demands and constant increase in the cost of production are the factor which have shaken the foundation of poultry sector in Pakistan (Pak. Poultry Feb. 1998). Another important factor affecting the poultry industry is the outbreaks of different diseases, which can have devastating effects particularly on intensive production. Thus recognition, treatment or prevention of disease is of crucial importance and is the subject of research and investigation (Gordon & Jordon 1985). Newcastle disease is one of the most common infection world-wide and also in our country.

The simplest and most logical control measure against Newcastle disease is to prevent transmission of virus to a susceptible bird. A second effective measure is through the vaccination, which gives the animal a degree of protection against infection in the events of exposure. Improved biosecurity and an awareness of the need for appropriate vaccination

programs, reduce the potential losses caused by both catastrophic and erosive infection in commercial-scale farm, village cooperative and in integrated operation (Shane 1997).

Newcastle disease, a respiratory disease of poultry, is caused by NDV (Avian parainfleunza type I virus) that can cause up to 100% morbidity and up to 80% mortality in five days in susceptible chicks . NDV can act in combination with other respiratory disease agents to cause a respiratory disease complex (Frank, 1981). Recently an outbreak of Newcastle disease has killed about two million birds (The Daily Dawn, December 28, 1997), as such epidemics are very common worldwide.

Effective vaccination is considered to be the most effective way to eradicate an infection. NDV vaccines may be expected to stimulate a substantial degree of immunity in a large population of healthy vaccinated bids. The Bl, LaSota, F, Roakin, Mukteswar and Haifa vaccines have been commonly used. It is emphasized that control over reconstitution of live vaccine is required to ensure potency. The actual administration of vaccine should be monitored by determining antibody titre. However, the infection has not been eliminated and remains widely distributed due to failure to vaccinate properly, failure of vaccine, or concurrent and debilitating disease.

Keeping in view the importance of vaccination as a preventive measure against viral infections specially Newcastle disease, a study was carried out to show the effectiveness of the Newcastle disease vaccines commercially available in the market- as live vaccine loses their efficacy by improper handling and storage etc.

MATERIALS AND METHODS

To study the efficacy of NDV vaccine, groups of chick were immunised with La Sota NDV vaccine (PRI Batch No.28/97) via intraocular route at different age and with single to three doses (given at one week interval) (Table-I). Chicks were sacrified according to the schedule shown in Table-I. Blood was collected in sterile tube and then serum was separated and stored in freezer.

Table-I
Grouping and Vaccination schedule

Group	Total	Number of chicks: Sacrified, No. of Weeks 0	1	2	3	4	Died, No. of Weeks 1	2	3	4	Remains at 5th W*
A-Non vaccinated	25	5	4	3	3	3	1	1	0	1	4
B-SD at 1 day old	20	0	4	2	2	2	1	2	1	1	5
C-1st D at 1 day+ 2nd D after 1W	12	0	0	2	2	2	0	0	0	2	4
D-1st D at 1 day+ 2nd D after 1W+ 3nd D after 2W	08	0	0	0	2	2	0	0	1	0	3
E-1st D after 1W	09	0	0	2	2	2	0	0	0	0	3
F-1st D after 1W+ 2nd D after 2W	08	0	0	0	2	2	0	1	0	3	
G-1st D after 1W+ 2nd D after 2W+ 3rd D after 3W	08	0	0	0	0	1	0	0	2	2	3
H-1st D after 2W	06	0	0	0	2	1	0	0	0	0	3
I- 1st D after 2W+ 2nd D after 3W	04	0	0	0	0	1	0	0	0	0	3
J- 1st D after 2W+ 2nd D after 3W+ 3rd D after 4W	03	0	0	0	0	0*	0	0	0	0	3

D=Dose W=Week SD=Single dose *=Blood was collected from wing from atleast two chicks

Note: Chicks died due to environmental factors but not with the Newcastle disease.

Detection of antibodies to Newcastle virus in the vaccinated and unvaccinated birds:

Antibody titre against NDV was determined by EliSA. Briefly 96- well ELISA plates

were coated with the NDV in coating buffer pH 9.5. After overnight incubation at 4°C, the plates were washed with washing buffer (saline containing 0.05% Tween-20). All the chicks' sera samples were diluted 1:2000 (Dilution was found suitable in other experiment not shown here) 100 µl of each sample in duplicate was applied on to the ELISA NDV-coated plates and incubated at 37°C in moist chamber for one hour. After proper washing, 100 µl of 1:2000 diluted Rabbit anti-chicken Ig (Prepared and standardised in our Laboratory) was added to each well. The plates were incubated at 37°C for one hour. The plates were washed after incubation. Sheep anti-rabbit IgG-HRP (supplied by Dr. Catty & C. Raykundalia of Department of Immunology, University of Birmingham, U.K.) was diluted 1:4000 and 100 µl was added to each well. After one-hour incubation at 37°C, the plates were washed OPD (10mg/25ml-substrate buffer) with few drops of H_2O_2 was prepared and 100 µl was applied on to each well. Plates were incubated for half an hour and then reaction was stopped by adding 50 µl of 20%H_2SO_4. Optical density was read at 490nm using an ELISA reader.

After five weeks all the remaining birds were challenged with a 10^6 ELD_{50} of virulent strain of NDV via intraocular route and chicks were observed daily for the sign and symptoms of disease or death for six days (5-6 days incubation period for the disease).

Table-2
Antibody titre against newcastle Disease Virus in the vaccinated and non-vaccinated birds measured by ELISA

	Optical Densities at 490 nm					
Group	24 hours	1 week	2 week	3 week	4 week	5 week
A	1.195	0.517	0.399	0.462	0.275	0.227
B	*	0.505	0.522	0.751	0.639	0.703
C	*	**	0.622	0.741	0.801	0.911
D	*	**	***	0.802	1.381	1.706
E	-	*	0.660	0.877	0.752	0.810
F	-	*	**	1.207	1.124	1.189
G	-	*	**	***	1.506	2.170
H	-	-	*	0.8	1.050	1.117
I	-	-	*	**	1.401	1.824
J	-	-	*	**	***	2.318

Number of vaccine dose with one week gap = * (single), ** (two doses), *** (three doses)
The average results of duplicate data from two representative experiment are shown here.

RESULTS

Chick groups were vaccinated at different ages (Table-I) and then antibody levels were determined by ELISA (Table-2). After following various schedule of vaccination, all the remaining chicks were challenged with the virulent NDV strain and survival of the chicks

were observed. Results are shown in Table 2 & 3.

Table 3
Protective immunity against virulent strain of NDV in the vaccinated and unvaccinated Chicks

Groups	Percentage of survived and dead chicks after challenging		
	Survived	Dead	Efficacy of the vaccine
A	0	100	-
B	60	40	60
C	100	00	100
D	100	00	100
E	100	00	100
F	100	00	100
G	100	00	100
H	100	00	100
I	100	00	100
J	100	00	100

The mean results of two representative experiments are shown here. The groups (B-J) were vaccinated with LaSota PRI vaccine according to the schedule shown in Table-1. Then all the remaining chicks (including non-vaccinated group A) were challenged with the 10^6 ELD_{50} dose of virulent NDV strain. Efficacy of the vaccine was determined as the percentage of survival birds in the vaccinated group.

Anti-NDV antibody levels of the vaccinated and nonvaccinated chicks were measured by ELISA. Control group (nonvaccinated) birds showed the presence of maternally acquired antibodies which gradually decreased with time (Table-2). Antibody titre rises in the vaccinated birds as compared with the nonvaccinated birds. Second dose and booster doses further enhance the immune response. Highest immune response was noted in the birds that were vaccinated at the age of one week.

At the end of experiment, all the chicks were challenged with the virulent strain of NDV through intraocular route. All the nonvaccinated birds died within five days (Table-3). Two of the chicks that received a single dose of vaccine at the age of 24 hours also died due to the Newcastle disease. However all the chicks that received NDV vaccine survived the challenged dose indicating protective levels of antibodies against NDV.

DISCUSSION

Poultry industry is under serious crisis due to ban on parties and also due to low rate of production. A large sum of money is being spent on disease control and prevention. High cost of poultry feed and medication adds to the misery of poultry farmers.

Pakistan poultry sector consumes medicine and vaccine worth of Rs.1068.39 million per annum (Fortnightly Reporter 1997). For a developing country this is a huge amount of money being spent on the poultry disease control. In poultry industry there is indiscriminate use of vaccine and antibiotics. This not only exert a negative effect on production but meat having these drugs is a health hazard for the poultry consumers. Secondly there is very poor or no facilities for storage of vaccine particularly live or attenuated vaccines, that may loose their efficacy at high temperature.

The study was conducted to study the effectiveness of Newcastle disease vaccination using commonly available LaSota vaccine in the market. The sera of vaccinated and nonvaccinated birds were analysed by ELISA for the levels of antibody titre.

LaSota NDV vaccine-PRI, available in the market, was found to induce a good response of antibody production. Antibody level was maintained after giving second dose and third dose. One of the groups received vaccine at one-week age, had high levels of antibodies that further increased after subsequent doses. One-day old chicks were found to have sufficient maternal antibody that persist up to three weeks. Hanson, 1978 reported that chicks up to 3-weeks of age have maternal antibodies that protect from virus challenge.

After vaccination schedule, chicks were challenged with the NDV virulent strain. All the nonvaccinated chicks died due to lack of immunity. Forty percent of the chicks that received a single dose of vaccine at the age of 24 hours died due to Newcastle disease. This may be due to the improper vaccination or there are maternal antibody that suppresses the immune response to the vaccine. However, second vaccine dose found to induce a protective immune response in the day-old vaccinated chicks (Table-3).

High levels of maternal immunity often inactive mild attenuated vaccine virus administered to chicks. This interference phenomenon is important in timing the first "priming" dose of vaccine which stimulate immunity against NDV. If first vaccine is administered too early in relation to the decline in maternal antibody, field challenge of susceptible birds will occur (Shane 1997).

Flock application at day old with attenuated vaccine is followed by one or more booster doses. For young breeder flocks, which are housed at high levels of biosecurity, the first vaccine may be delayed until 7-14 days of age of ensure active priming of the immune system.

A good correlation was observed between ELISA and challenge result used for comparison. Therefore ELISA can be used to evaluate a flock response to vaccination and for serological survey to detect NDV antibody in other hosts (Srinivasan *et al.*, 1992; Takes *et al.*, 1992).

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