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COMPARATIVE SERO EVALUATION OF LIVE AND KILLED NEWCASTLE DISEASE (ND) VACCINE IN BROILER FARM

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Abstract: Qualitative and quantitative measurements of antibody level in serum of vaccinated broiler flock (120 broilers), batch name-"broiler-43" of Usha Poultry and Feed Farm was performed in regard to compare the immunoresponse of both live and killed Newcastle Disease (ND) vaccine. Haemoagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA) tests were performed. To achieve this task, random serum sampling was done. The tests were performed at the Virology Laboratory of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, and Usha Poultry and Feed Farm Immunology Laboratory. Twelve serum samples were collected of which 10 were tested using both HI and ELISA tests, respectively. The average haemoagglutination count was 112 and ELISA titer was 3273 when flocks vaccinated at day 7. Tests were performed after 6 day of vaccine administration. The rest of the tests were performed 14 days after vaccine administration. The result in regard to immune response vaccinated at day 21 showed HI average 30 and ELISA average 598. The subsequent results when vaccinated at day 35 showed HI titer average 58 and ELISA titer average 2264. Analytical results when vaccinated at day 70 showed average HI titer 128 and ELISA titer 4311. When vaccinated at day 120, results showed HI titer average 480 and ELISA titer average 8122. The nature of immunoresponse was increasing at the beginning, decreasing at the mid-point and again increasing later on. Live vaccine showed short term high immunoresponse whereas, killed vaccine showed long term slow increasing immunoresponse. Further works on sero-response on molecular analysis of immunity would be worthy to investigate.

Key words: Live & Killed Newcastle Disease Vaccine, ELISA, Heamoagglutination Inhibition test.

Introduction

Newcastle Disease is a fatal and is controlled by effective vaccination. Control and eradication of the fatal disease (ND) require availability of quality vaccine and routine vaccination schedule. This is crucial for broiler chicken as well. Two types of vaccines against ND (viz. attenuated but live and killed) need evaluation for proper disease management and better production of poultry. In Bangladesh, poultry industry is one of the resourceful sectors to develop and meet-up the increased demand of protein.

Vaccine is one of the most effective way to prevent viral disease by triggering the defense mechanism of both humoral and cell mediated immunity (CMI) of birds. The main goal of vaccine is to protect the birds from specific pathogen (Phatak, 2000).

The objective to administer live vaccine is to establish an infection in the flock, preferably in each bird at the time of application (Goutebroze and Berg, 2000). When live vaccine is administered through appropriate route, it is multiplied inside the host and induces desired immunity, and the immunity lasts for short period (Phatak, 2000).

The main appeal of live vaccine is that, they may be administered by expensive mass application process (Goutebroze and Berg, 2000). For comparative evaluation of serum, immunological components like chicken serum is needed which is the source of antibody against Newcastle disease. Evaluation process includes wide range of immunological reaction based on antigen and antibody. Some tests (e.g. ELISA) show color due to break down of substrate and some test depends on agglutination of R.B.C. Haemagglutination Inhibition or HI Test is widely used for detection of Newcastle disease and is economical.

Newcastle Disease (ND) vaccination challenge model is a standard way to study the immunological functions. After injecting the vaccine to be tested and a subsequent vaccination with a live ND vaccine, both ND antibody titer and percentage of protection after a ND challenge require measuring. This is estimated from the comparative sero-responses and protection rate (Goutebroze and Berg, 2000).

Based on the above discussion, the present research work was undertaken with the following objectives:

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1. To determine the antibody responses in broiler flocks vaccinated with live and killed Newcastle Disease (ND) vaccine using enzyme linked immunosorbent assay (ELISA) and haemagglutination inhibition (HI) tests.
2. To compare the antibody responses between the two groups of chickens vaccinated with live and killed Newcastle Disease Vaccines, respectively.

Materials and Methods

The necessary equipments and chemicals used in the experiment was provided by the authority of Bangladesh Livestock Research Institute, Savar, Dhaka and Usha Poultry and Feed farm, Savar, Dhaka.

Serum: The experiment was performed on commercial broiler breed. Blood was collected and serum was isolated routinely from broiler farm. The flock name was "broiler-43". In the flock, whole 120 broilers was used for experiment.

Vaccine: The concerned vaccines were live and killed vaccine against Newcastle disease which were administered through intra-muscular injection and eye or nasal drops. The used vaccine trade name was clone-30 of Intravet Company, Holland. Live vaccine administration route was eye drop and killed vaccine route was intra-muscular injection (Table 1).

Collection of blood, preservation and test of serum sample: For experimental purposes, 12 serum samples were collected randomly from the flock "broiler-43" at different days, of which 10 samples were tested using ELISA and HI respectively. Extra 2 was used if any sample was disposed. Using separate disposable syringe, blood samples were withdrawn and kept in ice bag until shift to the laboratory.

Vaccination of broiler and test date of serum sample: Vaccination dates were designed according to the type of vaccination. Vaccination began from the timetable of vaccine administration followed in Usha Poultry and day of test is shown in Table 1.

Table 1. Vaccination dates with respective vaccine type, routes of administration and test day

Date of vaccination	Type of vaccine	Route of administration	Test date (days after vaccination)
7	Live	Eye/nasal drop	6
21	Live and Killed	Eye drop and intra muscular -injection	14
35	Killed	Intra muscular injection	14
70	Killed	"	14
120	Killed	"	14

Serological evaluation of NDV: The serum samples were collected at different intervals after different vaccinations and were tested by HI and ELISA tests.

Chick embryonated egg is suitable for antigenic (virus) culture. For this purpose, day 7 old embryonated egg from hatchery incubator was collected. The required eggs were 5 to 6. Virus from stock was inoculated in the allantoic fluid of the egg by syringe. From 1st to 5th day, virus infection was observed. When infection occurred successfully, it was detected (from the 1st day of infection) by visual observation. After that allantoic fluid was collected. The fluid was kept in eppendorf tube and was stored in deep freezer (-20°C) for about 12 hours. Then titer of the antigen fluid was determined.

Preparation of R.B.C. solution: Blood was collected from a healthy and disease free chicken. One-ml sodium citrate solution was used in syringe to prevent R.B.C. clotting while collecting bloods. 0.5 % R.B.C. solution was prepared by addition of 49.75 ml PBS solution with 0.25 ml of R.B.C., the resultant volume was 50 ml.

Haemagglutination Test (HA): NDV has a wide range of erythrocyte agglutination ability. The main prospect is that Haemagglutination (HA) is inhibited by specific antisera. HI and other testes were used to measure the immune status of vaccinated broilers. Two replications were done in HI.

Calculation of antigen titer: HA test was done for calculation of antigen titer determination, which was used in the HI test. HA titer i.e. antigenic titer was determined in every test date as previous titer of antigen diminishes.

***Determination of the titer of antigenic solution*:** After performing haemagglutination test (HA), titer of antigen solution was performed. The working antigen solution of NDV was prepared as follows: The HA

result was the dilution reading, where, haemagglutination reading was divided by constant standard 8.

Each well of the micro titer plate needs 50 µl antigen solution.
Therefore, 96-well plate needs 4800 µl ≈ 5 ml antigen solution.
For safety, 1 ml extra solution was taken with the 5 ml solution. The derivation of the above equation was then used to divide total quantity of solution i.e. 6 ml for 96-well plate to obtain required antigen quantity. The resultant was the quantity of antigen for one plate. Final working concentration was made by mixing of distilled water (DW) and quantity derived antigen solution in 6:1 dilution and it was taken for safe operation of the experiment for 3 plates (96-well).

***Haemagglutination Inhibition Test*:** Haemagglutination pattern of HI test at day 7 vaccinated serum sample has shown in fig.-2. Various stages involved in HI tests are following: serum dilution, antigen inoculation, R.B.C inoculation and reading of haemagglutination. Column 11 is positive and 12 is negative control well.

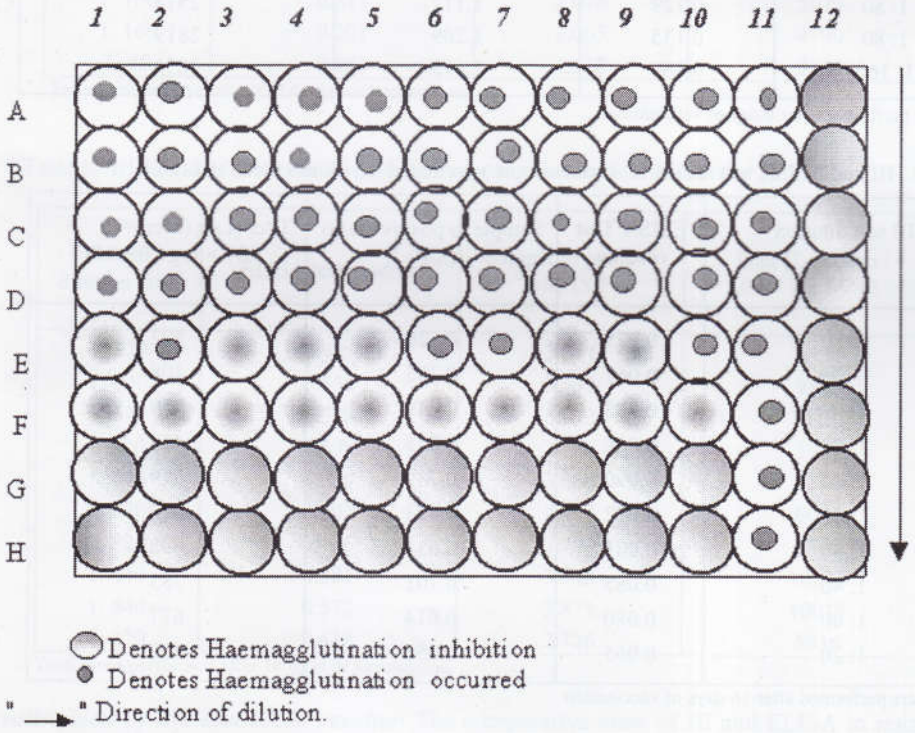

Fig. –1. Schematic presentation of day 7 vaccinated broiler serum sample.

Enzyme Linked Immunosorbant Assay (ELISA): The standard protocol was followed IDEXX laboratories (2001) regarding ELISA test.

Results and Discussion

A wide range of immunological reactions occurred in the tests. HI test of the serum sample was performed using ELISA test for the same serum sample. Different test results according to vaccination dates are presented in Table 2 to 6. In these Tables, HI dilution point counts and respective ELISA test computer reading has shown. In case of ELISA, sample to positive ratio is denoted by S/P. Separate positive control mean and negative control mean is also shown for specific ELISA test result determination. After obtaining the results, a further statistical and logarithmic calculation (Table 7) was done and compared for live and killed vaccine immune response.

Comparative pictures of HI and ELISA tests are shown in Table 7. The average haemoagglutination count was 112 and average ELISA was 3273 vaccinated at day 7. The analysis of immune response vaccinated at day 21 showed HI average of 30 and ELISA average of 598. The subsequent analysis (vaccinated on day 35) showed that HI titer average was 58 whereas ELISA titer average was 2264. Analysis on vaccinated at day

70 results showed that average HI result was 128 while ELISA test average was 4311. Vaccinated at day 120 results showed HI titer average 480 and ELISA titer average 8122.

Table 2. HI and ELISA test results of serum vaccinated (live) at day 7

HI Test dilution ratio	ELISA Test reading	Sample to positive ratio (S/P)	Titer [Log (titer) = 1.09 × log S/P+3.96]
1: 80	0.142	1.322	3107
1: 160	0.162	1.645	3941
1: 80	0.139	1.274	2983
1: 80	0.128	1.096	2533
1: 80	0.131	1.145	2655
1: 160	0.162	1.645	3941
1: 160	0.171	1.790	4322
1: 80	0.129	1.112	2574
1: 80	0.135	1.209	2819
1: 160	0.160	1.612	3857

Tests were performed after 6 day of vaccination.

Table 3. HI and ELISA test results of serum sample vaccinated (live and killed) at day 21.

HI test dilution ratio	ELISA Test reading	Sample to positive ratio (S/P)	Titer [Log (titer)= 1.09 × log S/P+3.96]
1: 20	0.075	0.526	572
1: 20	0.067	0.385	408
1: 40	0.082	0.649	719
1: 20	0.068	0.225	428
1: 40	0.090	0.789	819
1: 20	0.072	0.473	510
1: 40	0.081	0.631	698
1: 40	0.085	0.701	783
1: 40	0.080	0.614	677
1: 20	0.065	0.350	368

Tests were performed after 16 days of vaccination

Table 4. HI and ELISA test results of serum sample vaccinated (killed) at day 35

HI test dilution ratio	ELISA Test reading	Sample to positive ratio (S/P)	Titer [Log (titer)= 1.09 × log S/P+3.96]
1: 80	0.132	1.403	3314
1: 40	0.099	0.824	1856
1: 40	0.090	0.666	1472
1: 20	0.062	0.175	343
1: 40	0.096	0.771	1727
1: 80	0.133	1.421	3360
1: 80	0.136	1.473	3495
1: 80	0.131	1.385	3269
1: 80	0.113	1.070	2466
1: 40	0.087	0.614	1346

Tests were done 14 days after vaccination

Table 5. HI and ELISA test results of serum sample vaccinated (killed) at day 70

HI test dilution ratio	ELISA test reading	Sample to positive ratio (S/P)	Titer [Log (titer)= 1.09 × log S/P+3.96]
1:160	0.213	2.097	5137
1:160	0.233	2.315	5720
1: 80	0.141	1.315	3088
1:80	0.139	1.293	3032
1:160	0.208	2.043	4992
1:160	0.203	1.981	4847
1: 80	0.130	1.195	2783
1: 160	0.211	2.076	5079
1: 160	0.201	2.065	4790
1: 80	0.161	1.532	3648

Tests were performed after 14 days of vaccination.

Table 6. HI and ELISA test results of serum sample vaccinated (killed) at day 120

HI test dilution ratio	ELISA test reading	Sample to positive ratio (S/P)	Titer [Log (titer) = 1.09 × log S/P+3.96]
1: 320	0.421	2.581	6439
1: 640	0.554	3.717	9585
1: 640	0.578	3.923	10163
1:320	0.398	2.384	5907
1: 640	0.539	3.589	9226
1: 320	0.403	2.427	6022
1: 320	0.436	2.709	6789
1: 640	0.581	3.948	10236
1: 640	0.572	3.871	10019
1: 320	0.438	2.726	6836

Tests were performed after 14 days of vaccination.

Comparative view of live and killed vaccine: The comparative view of HI and ELISA in regard to live and killed vaccine response has shown in Fig.-2. The figure view recommends the nature of titer for both HI and ELISA test. In Table 7, Log titers of HI were 2.04, 1.47, 1.76, 2.10 and 2.68 and log titer of ELISA were 3.51, 2.77, 3.35, 3.65 and 3.90 respectively. After plotting the above points, two 'V' shaped curves obtained. The curve for ELISA lies upward of HI.

Table 7. Average of HI and ELISA test results

Serial no. of test for HI and ELISA	Day of sample tested	Average titer of HI test (dilution)	Average titer of ELISA test	Log titer of HI	Log titer of ELISA
1	7	112	3273	2.04	3.51
2	21	30	598	1.47	2.77
3	35	58	2264	1.76	3.35
4	70	128	4311	2.10	3.63
5	120	480	8122	2.68	3.90

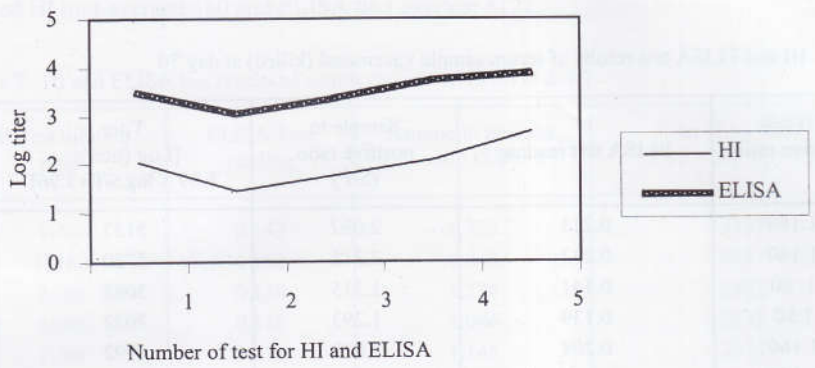

Fig.-2. Comparative view of live and killed vaccine response using HI and ELISA

Immune response against live vaccine was high in titer for both cases of HI and ELISA tests vaccinated at day 7. Tests were performed after 6 days of vaccination to observe the immediate response to immunity against live vaccine. Average result represents that, the live vaccine took shorter period to develop desired immunity. The study reveals that immunity grown within 6 days of vaccine administration was satisfactory.

The immune response vaccinated at day 21 shows reduced immunity compared to previous tests. This is may be due to administration of both live and killed vaccine together or interference of the two-vaccine (Box and Ellis, (1985). The subsequent average results represent that, administration of killed vaccine has longer period effects on immunity than live vaccine. The time requirement to grow desired immunity for killed vaccine is longer than live one. Killed vaccine found to increase the immunity up to120 days.

In HI test, few sample serums showed decreased haemoagglutination count compared to other serum samples of the same day e.g. at 1:20 dilution haemagglutination was found at day 35 which is less than other of the same date (Table 4). For ELISA, similar result was found as titer 368 at day 21 (Table. 3). The possible causes of such results might be non-vaccinated of the specific birds, loss of vaccine efficacy and viability, test errors, environmental effects etc. However, the average result of HI was satisfactory. Other limitations were high room temperature and short duration of experimental period etc.

Conclusion

As a whole if we consider the nature of immune response, we can find that it was increasing at the beginning and decreasing at the mid-time and was again increasing later on. ELISA results were not satisfactory as single sample was dispensed in place of duplicate sampling. The experimental investigation shows that overall results were satisfactory, which coincides with that of Phatak, (2000), who also expressed the similar nature of immunity for live and killed vaccine.

Based on the above investigation, it is advisable that poultry vaccination must be based on the immune status of broiler flock to reduce the production cost. Live and killed vaccine should not be used together. Further developments on this areas of concern emerges use of molecular biology technology or Biotechnology tools of research which enables a much greater understanding of pathogenicity (Rott and Klenk, 1988) and antigenicity (Russell, 1988). NDV has enabled most closely involved cloning of gene(s) (Miller and Emmerson, 1988). It is therefore, possible to speculate on the use of cloned genes expressed in vectors or sub unit vaccines for ND. Several groups of scientists are working along these lines of investigation at present. Chickens might be immunized by infecting with recombinant vaccinia virus expressing NDV F-glycoprotein (Meulemans, *et. al.*, 1988). Further experimental investigation using innovative tools of molecular biology at different poultry farm levels would be worthy to investigate.

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