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Humoral Immune Response to Newcastle Disease Vaccine (Lastoa Strain) in Broilers

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Abstract: This research was carried out at the Department of Microbiology, University of Agriculture Faisalabad, Pakistan in order to study humoral immune response to Newcastle disease vaccine (Lastoa strain) in broilers. For this purpose, 30-day-old broiler chicks were procured from Olympia chicks. The chicks were divided into three groups containing 10 chicks each. The HI-Ab-titre against ND virus in the control group showed an overall Ab-titre with GMT=3.99.The HI-Ab-response in broilers chicks with single dose of Lastoa vaccine ranged from 1:8 to 1:32.This group showed an overall HI-Ab- titer with GMT=13.931. While the HI-Ab titer against ND virus was found to be in the range of 1:16 to 1:128 in broilers chicks which were vaccinated with the 1st dose of ND virus (LaSota strain) at the age of 2 weeks followed by a booster dose with the same strain at 3 weeks of age. The overall HI-Ab-titre against LaSota strain of ND virus came out to be GMT=55.692. During present investigation a significant increase in HI-Ab-titre (GMT) against ND virus was observed in broilers from single to boostered vaccination group.

Key words: Humoral immunity, ND vaccine, Lasota strain, Olympia chicks

Introduction

Newcastle disease is highly contagious. All birds in a flock usually become infected within three to four days. The virus can be transmitted by contaminated equipment, shoes, and clothing and free-flying birds. During the active respiratory stage, it can be transmitted through the air. The virus is not thought to travel any great distance by this method. Recovered birds are not considered carriers and the virus usually does not live longer than thirty days on the premises. Vaccination is practiced widely and is the recommended method for prevention. Several types of vaccines are available but the most successful and widely used is the mild live virus vaccine known as the B_1 and La stoa types. The vaccines may be used by drops into the nostril or eye, addition to the drinking water or applied in spray form.

Newcastle disease (ND) is an acute paramyxoviral infection to the poultry birds and is worldwide in distribution. The only mean to control this malady is through prophy- lactic vaccination. A number of studies have been conducted to evaluate different vaccines against ND in order to find out their safety, efficacy and reliability. In many of the viral infections humoral and cell mediated immune responses play a pivotal role in protection against such diseases (Kumar *et al*., 1988). Both humeral and cell mediated immune responses are essential for complete protection (Chandrasekar *et al*., 1989). Unfortunately previously no study has been carried out in this country to evaluate the humoral immune response against LaSota vaccine strain of ND virus in broiler chicks by hemagglutination inhibition (HI) test. Therefore the present research study was undertaken to study the humoral immune response against Lasota vaccine strain of ND virus in broiler chicks using hemagglutination inhibition (HI) test.

Materials and Methods

Experimental animals: Thirty, day-old broiler chicks were procured from Olympia chicks, Faisalabad kept at the Department of Veterinary Microbiology, University of Agriculture, Faisalabad. The chicks were provided feed and water and were divided into three groups as; Group A: Comprised of 10 chicks. Vaccinated with commercial Newcastle disease (ND) vaccine (LaSota strain) through drinking water at the age of 14 days. Blood samples were collected aseptically 14 days after vaccination. GROUP B: Comprised of 10 chicks, vaccinated with LaStoa vaccinal strain of ND virus at the age of 14 days through drinking water and a booster doses was given after 7 days through drinking water. Blood samples were collected after 14 days post booster vaccination. GROUP C: Comprised of 10 chicks and was kept as control to obtain peritoneal macrophages.

Vaccine: Freeze-dried ND vaccine (LaSota strain) containing 1000 doses was purchased from the local market and placed at 4°C till use.

Reconstitution of vaccine: A 1000 doses vial containing of LaSota strain (Pliva Zagreb Croatia Batch NO.

Table 1: Conversion of base - two logarithmic mean titers to geometric mean titers (GMT)

Mean Titer A			Reciprocal Of GMT at proportionate distance between dilutions									
1/5	1/10	1/20	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
1	0	0	5	5	6	6	7	7	8	8	9	9
2	1	0	10	11	12	12	13	14	15	16	17	19
3	2	1	20	21	23	25	26	28	30	32	35	37
4	3	2	40	43	46	49	53	57	61	65	70	75
5	4	3	80	86	92	98	106	113	121	130	139	149
6	5	4	160	171	184	197	211	226	243	260	279	299
7	6	5	320	343	368	394	422	453	485	520	557	597
8	7	6	640	686	733	788	844	905	970	1040	1114	1194
9	8	7	1280	1372	1470	1576	1689	1810	1940	2079	2224	2389
10	9	8	2560	2744	2941	3152	3378	3620	3880	4159	4457	4777
11	10	9	5120	5487	5881	6303	6756	7241	7760	8317	8914	9554
12	11	10	10240	10975	11763	12607	13512	14482	15521	16635	17829	19109
13	12	11	20480	21950	23525	25214	27024	28963	31042	33270	35658	38217
14	13	12	40960	43900	47051	50428	54047	57926	62084	66540	71316	76434
15	14	13	8920	87800	94101	100856	108094	115852	124168	133079	142631	152868
16	15	14	163840	175599	188203	201711	2161188	231705	248335	266159	285262	305736

A Mean of titration endpoints expressed by dilution or tube number. Dilution of test material (for example, Serum) in first tube of twofold series. For essays with an initial dilution of ½, use the 1/20 column and divide results by 10.

2414082) was suspended in 10 ml were added to 500 ml water, making a standard dose for 30 chicks. Out of 20 chicks, 10 chicks were revaccinated after 7 days of first vaccination in the same way (drinking water).

Haemagglutination inhibition (HI) test for humoral immunity

Collection of serum samples: 3-5 ml of blood was collected aseptically from wing vein of all the experimental birds in standard test tubes. These samples were kept undisturbed for 15 minutes for clotting. Clots were broken with the help of a blunt glass jet. The tubes were centrifuged at 1500 rpm for 5 minutes and then serum was collected and stored in small vials at -20°C. Serum was heat inactivated at 56°C for 30 minutes in a water bath. The serum samples were then processed for HI antibody titration according to the method described Allan *et al.* (1978).

Washing of erythrocytes: 5ml blood was collected from an adult bird in a test tube containing 0.5 ml of a 4 per cent sodium citrate as anticoagulant. The blood was centrifuged at 1500 rpm for 5 minutes and the supernatant (plasma) and buffy coat were discarded. Packed RBCs wee resuspended in 10 ml of phosphate buffer saline (PBS).The process of RBCs suspension in PBS and centrifugation was repeated until a clear supernatant was obtained. The Packed RBCs were resuspended in a measured volume of physiological saline solution to make 1 per cent RBCs suspension.

Determination of 4 haemagglutination units (4 HAU): 1000 doses vial of LaSota Newcastle disease vaccine was suspended in 5 ml of sterile physiological saline solution. 2-fold serial dilution of the vaccine was prepared micro titration plate with 25 ul of sterile physiological saline solution in each well.25 ul of washed chicken RBC (1%) were added into each well. After gentle mixing, the plate was left at room temperature for 30 minutes. The highest dilution causing haemagglutination (HA) was taken as one HA unit. The dilution resulted after dividing the one unit dilution of the virus by 4, was prepared with the ratio to that of 4 HA unit.

Determination of HI antibody titer: Titretek micro titration plates with 8 rows and 12 columns of wells which Allow 8 samples to be titrated were used. All the wells were filled with 25 µl of physiological saline solution with the help of four-channel micropipette except the first well of each row in which serum samples were titrated.

With the help of 4-channel micro pipette, 25 µl of each serum sample was transferred from first row of wells to second and so on thus diluting each serum sample as 1:2, 1:4,1:8,1:16: 1:32...1:2048.

After diluting the serum sample, 25 µl of 4 HA units of virus were dispensed in each well with the help of 4 channel micropipette and plates were left undisturbed at room temperature (25°C) for 30-minutes. 25µl of 1 percent washed chicken RBCs were added into each well and the plates were gently tapped to ensure even mixing. A 25µl physiological saline solution and 25µl of 1 percent washed chicken RBC were added into two of another plate to serve as control. The plates were kept undisturbed at room temperature (250°C) for 30 minutes until a clear pattern of haemagglutination of HI was seen.

Maximum dilution of each serum sample causing HI was taken as the endpoint. The HI titer was expressed as reciprocal of the dilution of serum in well taken as endpoint. The results of HI titer of the sera samples were recorded and subjected to statistical analysis to calculate the geometric mean HI antibody titer.

Table 2: Haemaggluatination Inhibition (HI) antibody titres (GMT) in broiler Chicks vaccinated with LaSota strain of ND virus

Group/Total No. of birds	HI antibody titers							
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	GMT
Control group (10)	4	2	4	-	-	-	-	3.99
Group A (10)	-	-	4	4	2	-	-	13.931
Group B (10)	-	-	-	1	2	5	2	56.692

Analysis: The geomean HI antibody titres of all the sera in each group were determined, according to the method described by Burg (1978). Tube number recorded the end dilution points of serological tests and any 2-fold-dilution series was used. The geomean HI titer was calculated by referring to a Table 1. The geomean HI antibody titer was calculated using 1:20 as original dilution the whole number (integer) was obtained from the average of tube number found in one of the column to the left of the Table 1. The decimal fraction was then found in the box heads of the rest of the table. If the dilution was 2-fold, then geomean Hi titer was divided by 10. The comparison of means and t-test of significance was applied using the methods described by Steel and Torrie (1980).

Results

Haemagglutination inhibition (HI) test: A total of 30 serum samples collected at different age from broiler chicks with single as well as double vaccinated groups showed different HI antibody titers. The LaSota strain of ND virus used in performance of HI antibody titer showed the 4HAU.10 broiler chicks (Control group) were randomly selected for the collection of serum sample. The HI-Ab-titre against ND virus in the control group showed an overall Ab-titre with GMT=3.99 (Table 2). The HI-Ab-response in this group ranged from 1:2 to 1:8 where 4 birds showed 1:2 HI Ab-titre, 2 birds showed 1:4 HI-Ab-titre and 4 Birds showed 1:8 HI-Ab-titre (Table 2).

Group A: Ten broiler chicks were vaccinated at the age of two weeks with laSota vaccinal strain of Newcastle disease virus. Serum samples were collected from these chicks at 4 weeks of age (14 days post vaccination) HI-Ab-response ranged from 1:8 TO 1:32. Where 4 birds showed HI-Ab-titre 1:8, 4 birds showed 1:16 and 2 birds showed 1:32. This group showed an overall HI-Ab- titer with GMT=13.931 (Table 2).

Group B: Ten broiler chicks were vaccinated with the 1st dose of ND virus (LaSota strain) at the age of 2 weeks followed by a booster dose with the same strain at 3 weeks of age. The serum samples were collected at 5th week, showed HI-Ab- titer ranging from 1:16 to 1:128 where a single bird, two, five and two number of birds showed HI-Ab-titre of 1:16, 1:32, 1:64 and 1:128 respectively (Table 2). The overall 1 HI-Ab-titre against LaSota strain of ND virus came out to be GMT=55.692. The HI-Ab-titre (GMT) against ND virus when compared with in the groups A and B by t-test of significance, it was found that there is a highly significant increase in HI-Ab-titers from single to boostered vaccination group.

Discussion

Humoral immune response: In the present study, 10 chicks at age of 14 days were vaccinated with LaSota ND vaccine. The HI antibody titre 14 days post single vaccination ranged from 1: 8 to 1: 32 with geomean HI antibody titre 13.9. Aerosol vaccination has been reported to induce higher levels of HI antibodies that drinking water and persisted for 11 weeks (Bacallao and Viamontes, 1988). Aerosol method has not been adopted in the present study and HI antibody titres may have persisted for 11 weeks because present study determines HI antibody titre 2 weeks post single vaccination. Similar results have been reported by Ratnaparkhe *et al.* (1981). Paulillo *et al.* (1987) reported that LaSota strain at 14 days of age produced best HI antibody titre which is in line with the present results.

The finding of the present investigation are well supported by the results of Akcadag *et al.* (1984) who observed that in chicks vaccinated with LaSota or B through drinking water, birds with log2 HI antibody titre of 28 remained healthy.In the present study, the HI titres of birds vaccinated with LaSota strain of ND virus through drinking water, 7 days post single vaccination ranged from 1: 16 to 1:128 with geomean HI antibody titre 55.692.

The results obtained during present investigation are in agreement with the results of Matuka *et al.* (1980) who reported a high antibody titre in birds previously vaccinated with Mukteswar or LaSota and revaccinated with LaSota strain of ND virus. The results of present study are in line with that of Giamborne (1981) who reported the greatest serologic response and best resistance to clinical ND in birds with low levels of maternal immunity at the time of first vaccination at one day and a booster vaccination at 20 days of age. In the present study, double vaccination was done 7 days after single vaccination.

The finding of the present study are coincided with the results of Chandrasekar *et al.* (1988) who reported a gradual increase in HI antibody titres after first vaccination and a large response to second dose. The

geomean HI antibody titre after single vaccination was 13.931 which reached at 55.692 after double vaccination.

From the present investigations, it was concluded that LaSota vaccine induces early immunity (GMT = 13.692 after 14 days of vaccination) and that to attain a higher HI antibody titres, a second vaccination 7 days post single vaccination is necessary.

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