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Evaluation of Recombinant Interleukin-2 on Humoral Immune Response of Newcastle Disease Vaccinated Chickens

[1]B.E. Shambulingappa and [2]R.D. Padalkar

[1]Department of Vety Microbiology, Veterinary College, KVAFSU, Shimoga, Karnataka, India
[2]Department of Vety Microbiology, Veterinary College, KVAFSU, Hassan, Karnataka, India

Abstract: Totally 120 chickens belonging to three groups (group-A, B and C) were used to study the effect of Rec IL-2 on specific immunity of Newcastle disease vaccinated chickens. Serum samples collected at 6th, 12th and 18th days post vaccination (F strain and R_2B strain) were subjected to HI test and SRID test to assess the specific immunity of chickens.HI test results of chickens vaccinated with R_2B strain of NDV revealed the titre values in group-A showing 1:256,1:256 and 1:512 in the samples collected at 6th, 12th and 18th days of post vaccination respectively. Whereas titre values in group-B were also in increasing trend, but remained at 1:256 titres. Group-C showed minimal titre values at 18th days of post vaccination (1:4).SRID test results revealed the readings of antibodies levels in group-A after vaccination with R_2B strain, raised up to 4 mg/ml in 18th day sampling, while group-C showed 2.5 mg/ml of antibodies even in 18th day of sample collection indicating immunomodulatory effect of Rec IL-2 in vaccinated chickens.

Key words: Humoral immune response · Recombinant IL-2 · Newcastle disease

INTRODUCTION

The poultry industry in India has emerged as the most dynamic and rapidly expanding segment of livestock economy as evident from the production level touching about 37 billion eggs and 791 millions tones broilers with a compounded annual growth rate of 8% and 15% respectively. Today India is the 4th largest egg producer and 5th in broiler (meat) production in the world with the production potential of 2.20 millions of eggs and 824 million tones of meat. Despite this achievement, the annual per capita consumption in India is only 36 eggs and 850 gms of poultry meat which is much lower, as compared to the world average of 124 eggs and 5.9 Kg meat. This is because of the high mortality and morbidity of the birds on account of dreaded diseases and is one of the major problem faced by poultry industry leading to heavy economic loss.Inspite of availability of effective vaccines against many of the diseases, problem still continues to pose a great threat to the poultry industry.

Among the various infectious diseases, Newcastle disease (ND) is one of the most dreadful viral diseases affecting chicken and continues to be a serious economic threat to poultry industry on account of increased flock mortality and morbidity and loss in egg production. Although there is tremendous development in the production technologies of conventional as well as newer generation of vaccines, complete protection against many of the diseases following vaccination could not be achieved because of innumerable causes of vaccine failure. To combat such vaccine failures, immunomodulators or immune response modifiers play a significant role that can revolutionize the field of immunobiology and vaccinology.

Interleukin-2 is the prototype member of a family of cytokines, among all cytokines; IL-2 has captured the attention of the researchers worldwide because of the central role played by this immune hormone and its receptor in the clonal proliferation of T-cells, B-cells and stimulation of Natural Killer Cell (NK) production. With the advent of Biotechnology and quantum leap research occurred in Recombinant DNA technology, now a day's Recombinant forms of IL-2 are available (commercial products), which can be administered to animals at the same time as they are vaccinated against a variety of organisms leading to increased level of protection. A plethora of articles have appeared on IL-2 of domestic animals, particularly in bovines. But lymphokinology of

Corresponding Author: B.E. Shambulingappa, Department of Vety Microbiology, Veterinary College, KVAFSU, Shimoga Karnataka, India. E-mail: shambu71@gmail.com.

chickens has received scanty attention and many aspect of it are still remained unexplored and studies on Recombinant IL-2 application as an adjuvant are scanty. Hence the present study is undertaken to study the humoral immune response in chicken treated with Recombinant Interleukin-2 and vaccinated against Newcastle Disease (ND).

MATERIALS AND METHODS

Materials and methods employed for studying humoral immune response in chicken treated with Recombinant IL-2 and vaccinated against Newcastle Disease.

Source of Birds: A total of 120 chicks (commercial chicks) supplied by commercial hatchery, Hospet will be used in the study.

Vaccine: The lentogenic and mesogenic vaccine of NDV, F and R^2B strain respectively will be used for vaccination. The F and R^2B strains will be procured from Institute of Animal Health and Veterinary Biological (IAH&VB), Bangalore.

Recombinant Interleukin-2: Commercially available Recombinant Interleukin-2 from Sigmalife sciences (USA) will be used for the present research work.

Vaccination Schedule: A total of 120 day old commercial layer chicks will be divided in to 3 groups, group-A, group-B and group-C comprising of 50 chicks each in group-A and B and 20 chicks in group-C. The chicks in group-A and B were vaccinated with 'F'strain of NDV by intra ocular and intra nasal routs on 7^{th} day and 4^{th} week, whereas with R^2Bstrain of NDV by S/c route on 9^{th} week. While group-C remained unvaccinated control.

Treatment with Recombinant Interleukin-2: The chicks in treatment group(Gp-A) will be administered with IL-2 at 2.0 µg/kg body wt by I/M route for four subsequent days after each vaccination, while group-B remained as vaccinated control.

Sampling from Experimental Birds: Blood: Heparinised blood (50 IU/ml) will be collected from wing vein from each bird after every vaccination on 6^{th}, 12^{th} and 18^{th} day in all groups. Also 10 ml of blood will be collected without anti-coagulant for serum after every vaccination on 6^{th}, 12^{th} and 18^{th} day in all groups.

Assay of Humoral Immunity:

1. Haemagglutination Inhibition (HI) Test:

Materials:

- Normal saline (0.85%).
- 0.8%chicken erythrocyte suspension.
- Micro plates.
- Dilutors and droppers.
- Serum samples.
- NDV-antigen-4HA.

Method: The micro test as per standard description [1] will be used for detection of HI titers from serum samples collected on 6^{th}, 12^{th} and 18^{th} days post immunization from birds. The HI test was done manually by B-procedure in 'U' bottom micro plates using diluters, droppers and 4 HA units of ND viral antigen.

Serial 2 fold dilution of serum in Normal saline is taken and 25µl per well 4 HA unit of antigen is added. Plates were incubated for 45 min at room temperature. 50 µl of 0.8%erythrocytes will be added to each well and plates will be incubated for one hr at room temperature before reading. The titer will be expressed as reciprocal of highest dilution of serum or higher inhibition of Haemagglutination.

Single Radial Immuno Diffusion (SRID):

Materials:

- Normal saline (0.85%) and 0.8% agarose.
- Water bath.
- NDV antigen.
- Well cutter and template.
- Coomassie blue stain.
- Serum sample.
- Whatman-paper.
- Scale and graphs.

Method

Preparation of NDV Antigens Agar Gels: Gel was prepared with agarose (0.8%) in normal saline. The agar diluents mixture was kept in a water bath at 45°C. One ml of NDV antigen heated to 45°C in a water bath and mixed with agar diluents mixture (5ml). Care will be taken to avoid bubbling and the total of 6 ml mixture will be delivered with a preheated pipette on to the glass slide of size 75mm×50mm.The antiserum agar mixture will be allowed to harden on a leveled table,4 rows of total 16 wells will be cut at 12mm apart in the agar layer, while placing the slides on a graph, with the help of a 3mm bore well cutter. The gel slides will be kept in a moist chamber at 4°C until used.

Performance of Single Radial Immunodiffusion Test: The specific antigen will be incorporated into agar as mentioned above, according to method of Mancini *et al.* [7]. The wells of the antigen agar gels filled with serum samples using a micropipette and incubated for 24 hrs in moist chamber at room temperature to read precipitin rings.

Staining of the Gel Slides: After incubation, the slides were washed for 2 days in normal saline and a day in distilled water. The slides were dried with a filter paper layered on slides. After the slides got dried, they will be stained with 0.5% of Coomassie blue stain for one hour and distained in a solvent containing one part of acetone, 4 part of water and 4 part of methanol. The diameter of the precipitin rings will be measured with the help of a scale.

RESULTS AND DISCUSSION

Haemagglutination-Inhibition Titres (HI): The samples collected from chickens at 6th, 12th and 18th day after each vaccination were subjected to HI test, the results were depicted in Table 1a and 1b and Fig. 1. Serum samples taken from chickens vaccinated with F strain on 7th day (I vaccination) were subjected for HI test and titres varying from1:128 to1:256 in group-A, from1:32 to1:64 in group-B, while HI titres of 1:4 detected in group-C (almost nil). Highest titre of 1:256 was shown in serum samples taken at 18th day post vaccination in group-A where IL-2 was given in addition to vaccination, where in 1:64 was the highest titre in case of group-B, where only vaccination was done.

Similarly in chickens vaccinated with F strain at 4th week, the titres were 1:128 to 1:512 in group-A from 6th, 12th and 18th days collection and showed reduced level of titres (1:32 to 1:128) in group-B. Minimal level of titres (1:4) observed in group-C, where no vaccination was done (unvaccinated control).

After III vaccination with R_2B (mesogenic) strain of NDV, the titre values were elevated in group-A showing 1:256,1:256 and 1:512 in the samples collected at 6th, 12th and 18th days of post vaccination respectively. Whereas titre values in group-B were also in increasing trend, but remained at 1:256 titres. Group-C showed minimal titre values at 18th days of post vaccination (1:4).

There was scanty literature available for comparing the results of present study to assess the humoral response against NDV with Recombinant IL-2 inoculations based on HI titres. The results in the present study were highly remarkable and significant that HI titres were increased up to 1:512 (group-A)in birds with RecIL-2 inoculation, where as titres were 1:256 (group-B)in birds without RecIL-2 inoculation, which was in agreement with the results of the study already conducted [8].

Table 1a: Haemagglutination Inhibition (HI) antibody titers

		TITERS		
Vaccines	Days of Sampling	Group A	Group B	Group C
I vaccination (F strain on 7th Day)	6	1:128	1:32	Nil
	12	1:128	1:64	Nil
	18	1:256	1:64	1:4
II vaccination (F strain on 4th week)	6	1:128	1:32	Nil
	12	1:256	1:64	Nil
	18	1:512	1:128	1:4
III vaccination (R_2B strain on 9th week)	6	1:256	1:64	Nil
	12	1:256	1:128	Nil
	18	1:512	1:256	1:4

Table 1b: Reciprocal values of Haemagglutination Inhibition (HI) titers

		TITERS		
Vaccines	Days of Sampling	Group A	Group B	Group C
I vaccination (F strain on 7th Day)	6	128	32	Nil
	12	128	64	Nil
	18	256	64	4
II vaccination (F strain on 4th week)	6	128	32	Nil
	12	256	64	Nil
	18	512	128	4
III vaccination (R_2B strain on 9th week)	6	256	64	Nil
	12	256	128	Nil
	18	512	256	4

Table 2: NDV antibody levels assessed by SRID in birds of different groups at I, II and III vaccination

Vaccines	Days of Sampling	Antibody concentration (mg/ml) Group A	Group B	Group C
I vaccination (F strain on 7th Day)	6	2.5	1.5	-
	12	2.5	1.5	-
	18	3.5	2.5	-
II Vaccination (F strain on 4th week	6	2.5	1.5	-
	12	2.5	2.0	-
	18	4.0	2.5	-
III Vaccination (R_2B strain on 9th week)	6	3.0	2.0	-
	12	3.5	2.0	-
	18	4.0	2.5	-

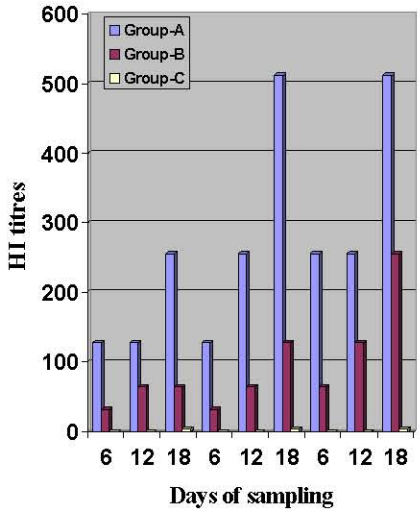

Fig. 1: Titres of HI antibody in chickens of Group-A, B and C after each vaccination

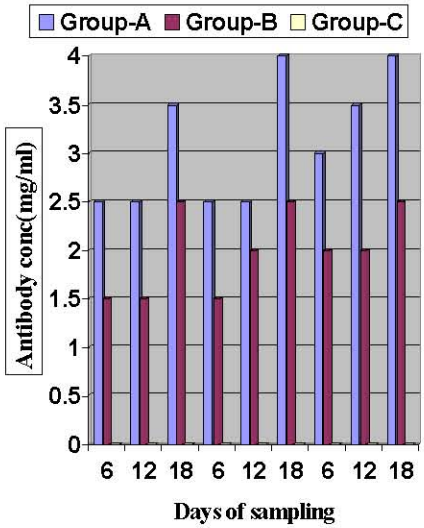

Fig. 2: Antibody levels of NDV assessed by SRID in chickens of group-A, B and C

Single Radial Immuno Diffusion Test (SRID): The samples collected from chickens at 6th, 12th and 18th day after each vaccination were subjected to SRID test, the results were shown in Table 2, Fig. 2. A standard curve was drawn on the graph using different concentration of antibodies against NDV i.e.2 mg/ml, 3 mg/ml and 4 mg/ml. The diameter of precipitation ring were plotted on X-axis and concentration of antibodies against NDV were plotted on Y-axis. A straight line was obtained and readings of the diameter of serum samples subjected to SRID were directly read on the straight line of the curve. The point where in the reading crossed the straight line was taken as the quantity of NDV antibodies in the serum. The results obtained in SRID for the quantum of antibodies in the serum were shown in table-2. Concentration of antibodies in serum samples collected at 6th, 12th and 18th days of each vaccination against NDV varied from 2.5 to 4.0 mg/ml in group-A, while it was 1.5 to 2.5 mg/ml in group-B and no detectable level of antibodies noticed in group-C.

In the present study SRID has been conducted as an additional tool for evaluating humoral immune response by assessing the antibody titres from serum samples collected at different intervals. Test procedure adopted by previous workers [2, 8] was employed in the present study. SRID results indicated that the birds in group-A had higher antibody levels in comparison to the birds in group-B, where only vaccination without Rec IL-2 inoculation was given, which was in agreement with the previous finding [8]. Inteleukin-2 as a potent adjuvant in vaccinated cattle has been demonstrated by many studies [4, 5, 9] and similar studies in guinea pigs [11], pigs [6] and also in mice [3]. The humoral immune response was evaluated in a study [10] on FMD vaccinated murine model to assess the role of recombinant human IL-2 as immunomodulatory molecule and IL-2 was able to enhance the specific immune response against FMD virus from mice receiving IL-2 along with vaccine as compared to mice administered with vaccine alone. Hence the present investigation on effects of Interleukin-2

on NDV vaccinated chickens has revealed a useful insight in to the immunomodulatory role of Recombinant IL-2 on humoral immune response in poultry birds and would act as an efficient adjuvant in poultry vaccines, thereby preventing excessive production loss of Poultry industry due to infectious diseases.

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