EFFECTS OF INFECTIOUS BURSAL DISEASE VACCINE ON THE IMMUNITY INDUCED BY NEWCASTLE DISEASE VACCINE IN BROILER BIRDS

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ABSTRACT: *Poultry farming in Pakistan has emerged as a profitable agro-based industry during the last two decades. However, the poultry development is being hampered by occurrence of various fatal infectious and non-infectious diseases. The Newcastle disease (ND) and Infectious bursal disease (IBD) are two major infectious diseases, which are continuously causing significant economic losses to the poultry farmers. A lot of vaccines had been introduced to control these diseases. However, failures encountered from time to time. The salient questions addressed in this study are to determine the interaction between commonly used vaccines against these diseases and its role in vaccination failure. A total of 250, day old broiler chicken were purchased and were randomly divided into 7groups; 35 birds in each group (A, B, C, D, E, F and G). The leftover 5 birds were used to detect the maternal antibody titer. Group A, B, C, D, E and F were vaccinated against ND and varying schedule of IBD. A total of 10 birds from each group were randomly selected for blood collection. The blood was collected from each bird and serum was prepared. The sera were analyzed by HI (Haemagglutination inhibition) test to detect antibodies against NDV and indirect ELISA for the detection of IBD antibodies.*

The results showed thatuse of IBDV vaccines has immunosuppressive effects. The immunosupression was evident by the lower humoral immune response to NDV vaccine detected by HI antibody titers. Immunosuppressive effect of IBDV vaccines also affects the weight of bursa, spleen and thymus and protection to biological challenge with virulent NDV.Considering the results of the present study it is recommended that intermediate strain of IBDV vaccine should be incorporated in the vaccination schedule

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1- INTRODUCTION

Poultry farming in Pakistan has emerged as a profitable agro-based industry during the last two decades. However, the poultry development is being hampered by occurrence of various fatal infectious and non-infectious diseases. The Newcastle disease (ND) and Infectious bursal disease (IBD) are two major infectious diseases, which are continuously causing significant economic losses to the poultry farmers.

ND commonly known as Ranikhet is highly contagious and highly fatal viral infection affecting many domestic and wild bird species globally [1]. It caused huge economic losses and has been engaging the attention of workers for its control. The severity of ND may vary from asymptomatic infection to highly fatal disease, depending upon the strain and tropism of the infecting virus, age of the bird concurrent infections and preexisting immunity against the virus in host bird at risk. The disease is caused by avian paramyxovirus serotype 1 (APMV-1) of the genus Rubulavirusbelonging to the subfamily Paramyxovirinae, family Paramyxoviridae, order Mononegavirales[2]. The virus is existing in the environment in three pathotypes i.e. Velogenic, Mesogenic and Lentogentic[3]. The disease is mainly controlled by vaccination.

IBD is an acute highly contagious immunosuppressive viral infection of young chicks [4]. Causative agent of IBD belongs to family Birnaviridae and genus Avibirnavirus[5, 6]. The outbreaks of the disease were reported in many parts of the world [7, 8, 9, 10].The incubation period of IBD virus is 2-3 days after exposure. In full susceptible flocks the disease appears suddenly and there is high morbidity rate usually approaching 100% while mortality may be nil but can as high as 20-30%. Many infection are silent owing to age of birds (< 3 weeks old), infection with avirulent field strains or infection in the presence of maternal antibodies [6]. The concern of the disease is the immunosuppressive activity of the IBDV resulted from severe damage of bursa of Fabricius[11, 12]. The immunosuppressive effects of IBDV had previously been reported to adversely affect vaccination against ND [11, 13, 14] and other viral infections [15, 16]. Therefore, the present project was planned to explore the immunosuppressive effects of IBD vaccineon ND vaccination.Therefore, the major purpose of this investigation is to study the immunosuppressive effects of IBD vaccines schedule being used in Pakistan on the ND vaccine.

2- MATERIALS AND METHODS

Total 250, one-day-old broiler chickens were purchased from the M/S Big Birds poultry breeders, Lahore. The chickens were reared in thoroughly cleaned anddisinfected experimental rooms of Microbiology Department, University of Veterinary and Animal Sciences, Lahore. The

chickens were offered feed and water *ad libitum* and were kept.At 1st day, birds were randomly divided into 7groups; 35 birds in each group (A, B, C, D, E, F and G). The leftover 5 birds were used to detect the maternal antibody titer. Group A, B, C, D, E and F were vaccinated according to schedule given in table 1 whereas group G was used as control.

Table 1: Vaccination programme of broiler chicken

No.	Group	IBDV vaccine	NDV vaccine
1	A	9th& 21th day	5th& 24th day
2	B	9th& 21th day	5th& 24th day
3	C	9th& 21th day	5th& 21th day
4	D	5th& 24th day	5th& 24th day
5	E	1st& 21th day	5th& 24th day
6	F	--	5th& 24th day
7	G	Control (un-vaccinated)	

2.1-Vaccination against other diseases

The birds in the various treatment groups were also vaccinated against Hydro-pericardium virus. The Hydro-pericardium vaccine was carried out through subcutaneous route on the 18th day of age (Hira Pharmaceutical)

2.2-Sera samples

At the first day a total of 5 experimental chicks were sacrificed to collect sera for detection of maternal antibody titer. Thereafter Blood sample were collected from all the groups on weekly basis up to 42nd day of life in order to determine the pre and post vaccination titers. A total of 10 birds from each group were randomly selected for blood collection. The blood was collected from each bird separately in disposable syringe and allowed to clot in slanting position at room temperature for separation of the serum. The sera then collected were stored at -20 0C till use.

2.3-Haemagglutination (HI) antibody response of chickens

Serum HI antibody response to NDV of the birds administered ND LaSota using Haemagglutination inhibition test [13].

2.3.1-Washing of RBCs for detection of Haemagglutination **virus**

Chicken whole blood was obtained in a beaker and mixed with anticoagulant (EDTA 1mg/ml). Blood was then poured in a centrifuge tube and centrifuged at 2000rpm for 3 minutes. The plasma and buffy coat was discarded, transferred without disturbing the sediment with the help of a Pasteur pipette. Red blood cells (RBCs) left at the bottom were re-suspended in physiological saline (8.5 % aqueous solution of sodium chloride) by gentle shaking. Re-suspended RBCs were again centrifuged twice. After final washing the pelleted RBCs were re-suspend to form 5% suspension of washed RBCs

2.3.2-PlateHaemagglutinationtest procedure

The test was carried out as described by Beard, (1989). Fifty micro liter of the saline solution was added in each of the 12 wells in a row A& B of 96 well immunoassay plate (96 round bottom micro titration plate: Kartel, Italy). In the first column of each series, 50ul of the virus suspension was added and mixed thoroughly. Fifty micro liter of diluted virus (1:2) suspension was transferred from the first well to the second well and mixed. This process was repeated till 11th well, from which 50 ul of diluted virus solution was discarded. No virus suspension was added to 12th well. To each well 50ul of 1% RBCs suspension was added and the plate was incubated for 30 minutes. Haemagglutination titer of the virus was the highest dilution showing complete agglutination.

2.4- Interpretation

Serum Sample with S/P ratio less than or equal to 0.2 considered negative ,S.P ratio greater than 0.2 (titer greater than 396) considered positive and indicate vaccination.

2.5- Calculations

1. Negative control mean (NCX) well A1 A(650)+ well A2 (A650) = NCX
2. Positive Control mean (PCX) well A3 A(650)+ well A4 A(650) = PCX
3. S/P Ratio = $\frac{\text{Sample mean-NCX}}{\text{PCX} - \text{NCX}}$
4. Titer- Relates S/P at a 1:500 dilution to an end point titer:Log_{10} Titer = 1.09 (Log_{10} S/P) +3.36

2.6-Lymphiod organs weight

Various lymphoid organ e.g. bursa of fabricius, thymus and spleen will be weighed at termination of experiment i.e. 42nd day of age. These organs will be removed, cleared off fat and tissue debris and then weighed [17].

2.7-Virulent NDV challenge of experimental chickens

Velogenic local field NDV isolate obtained from Department of Microbiology, University Of Veterinary and Animal Sciences, Lahore, was used as challenge virus. All the groups including the control were challenged with $10^{5.0}$ LD_{50} of NDV on day 35 of their age. All the challenged birds and non-challenged chicks were kept under observation upto day 7 post challenges for the development of any clinical sign and mortality. All the dead and moribund chicks were necropsied and their post mortem lesions were recorded.

2.8-Statistical analyses

Data of various treatment groups was compared by analysis of variance and statistically significant difference among the various treatment means were determined using least significant difference test at 5% level of probability [18].

3- RESULTS AND DISCUSSION

3.1- Humoral immune response to NDV vaccine

The present study was designed and conducted to determine the effects of Infectious bursal disease vaccines and vaccination schedule on immunity induced by ND vaccine in broiler birds. The antibody titers against ND of the birds from various groups were determined by HI. The antibody titers as detected by HI in all groups of chicks following vaccination with ND and IBD are presented in table 2.

The maternal HI antibody titer against Newcastle disease virus was 256.0 (GMT) at day one. On day 7 the GMT of HI antibody titers against NDV of groups A, B, C, D, E, F and G were 238.9, 238.9, 274.4, 256.0, 222.9, 256.0 and 128.0, respectively. The highest GMT of HI antibody titer was recorded in group C (274.4) and the lowest GMT in group G (128.0).

On day 14 the GMT, HI antibody titer of groups A, B, C, D, E, F and G were 48.5, 42.2, 55.7, 68.6, 32.0,

294.1 and 52.0, respectively. Group F shows the highest HI antibody titer (294.1) which was vaccinated with ND only.The lowest HI antibody titer was recorded in group E (32.0). On day 21 the GMT, HI antibody titer of
On day 28 the GMT, HI antibody titer of groups A, B, C, D, E, F and G were 90.5, 59.7, 104.0, 64.0, 104.0, 181.0 and 2.3, respectively. The highest HI antibody titer was recorded in group F (181.0) than group E (104.0) and C (104.0). However, the lowest GMT, HI antibody titer was recorded in group G (2.3).
On day 35 GMT, HI antibody titers of groups A, B, C, D, E, F and G 137.2, 64.0, 128.0, 68.6, 111.4, 315.2 and 0.0, respectively. The highest HI antibody titer was recorded in groups A, B, C, D, E, F and G were 36.8, 36.8, 42.2, 48.5, 22.6, 274.4 and 19.7, respectively. The highest HI antibody titer was recorded in group F (274.4) and the lowest GMT, HI antibody titer was recorded in group G (19.7).
group F (315.2) and the lowest GMT, HI antibody titer was recorded in group G (4.3).
On day 42 the GMT, HI antibody titers of groups A, B, C, D, E, F and G were 445.7, 388.0, 512.0, 388.0, 477.7, 588.1 and 0.0, respectively. Among the vaccinated groups, F shows the highest HI antibody titer (588.1). The lowest antibody titer was recorded in group
B (388.0). However, the non-vaccinated group G had 0.0 HI antibody titer.

Table 2: Comparison of HI antibody titers (GMT).

Groups	Days indicating Mean ELISA Antibody Titers						
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
A	256.0	238.9	48.5	36.8	90.5	137.2	445.7
B		238.9	42.2	36,8	59.7	64.0	388.0
C		274.4	55.7	42.2	104.0	128.0	512.0
D		256.0	68.6	48.5	64.0	68.6	388.0
E		222.9	32.0	22.6	104.0	111.4	477.7
F		256.0	294.1	274.4	181	315.2	588.1
G		128.0	52.0	19.7	2.3	0.0	0.0

3.2- Post challenge mortality

The chicken of the both vaccinated and non vaccinated groups (n=15) were challenged with $10^{5.0}$ LD_{50} virulent NDV field velogenic isolate on 35th day of life. The birds were kept under observation for 7 days for recording the development of clinical signs and mortality. All the chicks showing signs of ND were consider as susceptible to challenge. The mortality record of the chickens following NDV challenge is presented in table 3.
The effected birds were dull and depressed. There was chalky green diarrhea, respiratory distress. The postmortem examination of the chicken that died after challenge revealed hyperemia and hemorrhages in proventiculus, intestine and caecal tonsils. The trachea and lungs were congested.

Table 3: Post challenge mortality record

Groups	Mortality (%)
A	6/15 (40%)
B	10/15 (66%)
C	5/15 (33%)
D	9/15 (60%)
E	8/15 (53%)
F	1/15 (7%)
G (control)	15/15 (100%)

Velogenic NDV challenge was administered intraperitoneally @ $10^{5.0}$ LD_{50}/ml.

4-DISCUSSION

Newcastle disease (ND) and infectious bursal disease (IBD) pose great hazard to poultry industry in many parts of the world. In Pakistan, a lot of vaccines had been introduced to control these diseases. However, failures encountered from time to time. The salient questions addressed in this study are to determine the interaction between the most commonly used vaccines against these diseases and its role in vaccination failure.
In the present study different strains of infectious bursal disease virus (IBD) vaccines were incorporated in vaccination schedule of broilers followed in Punjab, Pakistan. The immune profile of ND and IBD vaccines were studied to evaluate the effect of IBD vaccine strain on ND vaccine immune response. The parameters studied were immune response to NDV vaccine, body weight ratio of lymphoid organ such as bursa, spleen and thymus, feed conversion ratio (FCR) and protection to virulent NDV challenge.

4.1-Humoral immune response for NDV vaccine with varying strain of IBD vaccine:

It was well established fact that maternally derived antibodies (MDAs) are protective against ND infection [19]. The chicks used in the present study were procured from a well reputed commercial hatchery. The breeder flocks of this hatchery were maintained in controlled environmental houses and follow an intensive vaccination programme. The GMT of MDA titer for NDV detected by HI in the experimental chickens is also found to protective titer (256.0) [20].
At 5th day of age all the groups primed with ND or ND/IB vaccine except group G. The groups A and B had given ND/IB vaccine, while groups C, D, E and F received only ND vaccine. On 1st week, just after 2 days of priming the GMT for NDV antibodies dropped except in groups C (274.0), D (256.0) and F (256.0). The non-vaccinated group G showed significant drop in the titer as compared to the vaccinated groups. The group E had lower GMT than other vaccinated groups because of residual effects or interference of IBDV complex vaccine (Bursaplex), which was given to

this group at day one. The findings are in accordance to that of Faragher, *et. al* [11]. However, no significant variation among the groups was recorded. The group G had GMT for NDV vaccine was 128 which indicate the gradual decrease in maternal antibody titer. Allan *et al*, [19] that found similar results, with maternal antibody titers.

The groups A, B, C and D received intermediate strain of IBDV vaccine at 9th day. At 2nd week of life the highest GMT, HI antibody titer was recorded in group F which received only NDV vaccine [21]. The intermediate strain and complex IBDV vaccinated groups had lower GMT than only NDV vaccinated group titer for NDV. It reflects the interference of IBDV vaccine which causes the drop in serum antibody titer for NDV. Similar were observed at 3rd week of age before boosting. Yuguda *et al* [22] reported immunosuppressive effects of IBDV vaccine on immune response to NDV vaccine. The non-vaccinated control group showed decrease in maternal antibodies as it did not receive NDV vaccine. The gradual drop in maternal antibodies is an acceptable fact reported by Natour, *et al.*, [23].

According to the schedule used in Punjab, Pakistan, birds were again vaccinated for IBD and ND as a booster dose on 21st day and 24th day of age, respectively; except group G; which was non-vaccinated control. The groups A, C and E had given booster dose of intermediate strain while groups B and D receive hot strain of IBD vaccine. The serum antibody profile determined at 4th week of age indicated a marked rise in antibody titer for ND HI antibodies. However, the GMT titer of HI antibodies in groups A, C, and E were 90.5, 104.0 and 104.0 which was significantly higher than that of groups B and D which were 59.7 and 64.0, respectively. The GMT HI antibody titer for ND vaccinated control was 181.0. Kelemen *et al*.,[24] also reported the HI titers induced by ND vaccine decrease in inverse ratio to virulence of IBD vaccine strain. The pattern of rise in HI antibodies was similar at 5th week of age. In brief the groups vaccinated with hot strain had lower GMT of HI antibody titers than intermediate strain vaccinated groups. These findings are in accordance with the [11,14,19] It could be concluded that IBDV vaccines interfere with the NDV vaccine.

4.2- Effects of IBD vaccine on humoral immune response and body weight ratio of thymus, bursa and spleen:

The hot strain used in this study provokes more invasive and immunosuppressive response than the intermediate strain. The humoral immune response induced by hot strain IBD vaccine was inferior to intermediate strain vaccine after one week of boosting. However, immune response measured at 5th week of age i.e. after two weeks of booster dose were indicated higher antibody titers in hot strains boosted groups as compared with intermediate strain boosted groups. The decrease in bursal body weight ratio (BBR) in birds vaccinated with hot strain of IBDV was lower than that of the birds vaccinated with the intermediate and complex vaccine. However, the differences were non-significant statistically. The IBDV vaccinated birds had lower BBR than NDV vaccinated and non-vaccinated control birds. There was no significant ($P>0.05$) difference among the various groups. Yadin*et al.,* and Wood *et al.,* [25, 26 respectively] reported similar kind of immunosuppressive effect of IBD live virus vaccine. Since the primary site of infection and inducement of lesion by IBDV is bursa of fabricius, the effects on the immune system may be significantly suppressive. The immunosuppressive effects are attributed to the depletion of B cells which become productively infected by the virus and are later destroyed to release the infectious virions. Degree of destruction and depletion of B cells which is directly related to the virulence and invasiveness of the strain of viruses also result in the correspondingly decrease in bursal weight and size.

The spleen body weight ratio (SBR) and the thymus body weight ratio (TBR) of groups A, B, C, D and E were slightly lower than the SBR and TBR of the control group F and G. However, no significant difference ($P>0.05$) was recorded among various groups. This was because the IBDV produce slightly swelling of spleen and thymus which resulted in pathological changes within these organs. But these damages were less extensive in both of the organ, than in the bursa of fabricius. These finding were agreement with the observation of Rinaldi, *et al.* [27].

4.3- Protection to biological challenge with virulent NDV:

It was observed that IBDV vaccine treated chicks have significantly higher mortality than the only NDV vaccinated chickens. Ezeokoli*et al*., (1990) [28] also reported that significantly higher mortality rates were observed in birds vaccinated with IBD vaccine than unvaccinated birds. The immunosuppressive effects of IBDV had adversely affect vaccination against ND [29]. The hot strains used in this study suppress antibody response to ND vaccination and protective vaccinal immunity. In contrast, birds that received intermediate strain were well protective from virulent NDV challenge [30].

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