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DETECTION OF MATERNALLY DERIVED ANTIBODIES (MDA) TITER AND COMPARISON OF INTERMEDIATE AND INTERMEDIATE PLUS (GM-97 STRAIN) VACCINES OF INFECTIOUS BURSAL DISEASE VIRUS

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ABSTRACT

The Infectious Bursal disease (IBD) is an important devastating disease among the infectious diseases of poultry in Bangladesh. Hence, this study was designed to determine the MDA titer and compare the two commercially available vaccines strains of IBDV ("GM-97 strain Intermediate plus" and "Intermediate type strain"). In this study, a total of 1500 layer birds were equally allocated into three groups (group-A, group-B and control). The Group-A and Group-B were vaccinated by "GM-97 strain Intermediate plus" and "Intermediate type strain" of IBD vaccine, respectively and control was unvaccinated. Blood samples were collected prior to vaccination (1- and 7-days old birds), as well as 7, 14, and 21 days after vaccination. The antibody titer was measured by iELISA test. The highest MDA mean titer was 6227.69±327.63 in day-old birds. The group-A birds had the significantly ($p<0.01$) higher antibody mean titer than group-B and control. The highest antibody mean titer was 9121.94±657.05 at the age of 39 days in group-A. The MDA titer at 1 days-old had the higher effect size (4.10; CI:4675.36-6072.01; n=16). In group-A, the highest effect size (4.49, CI:5953.80-7556.08; n=16) was in 32 days-old (14 d.p.v.) and the group-B had the highest effect size (3.35; CI:4861.04-6702.59; n=16) was in 32 days-old (14 d.p.v.). Significantly ($p<0.01$) higher histopathological-lesion scores were 4.75±0.25 and 3.5±0.65 in 32- and 39-days-old respectively in group-A. In brief, the protective level of IBDV MDA titer may remain up to 1 week of post-hatching and the Intermediate plus vaccine can generate higher antibody titer than the intermediate type.

Keywords: IBD, ELISA, Antibody Titer, MDA, Vaccine

INTRODUCTION

Poultry is one of the faster growing and important subsectors that has generated huge employment opportunity, playing a vital role in the reduction of poverty, and malnutrition in both urban and rural areas of Bangladesh (**Hamid *et al.*, 2016**). There are several constraints that hinder the development process in poultry sector; among them, disease is the major one. The flourishing poultry industry is indorsing a series of problems due to outbreak of infectious and non-infectious diseases, resulting the high mortality which brings huge economic losses in Bangladesh (**Hossain *et al.*, 2015**). Among the infectious diseases of poultry, the Infectious Bursal disease (IBD) is one of the important overwhelming diseases in Bangladesh (**Rahman and Samad, 2005**). IBD, a highly contagious acute viral disease that affects growing chickens and commonly known as Gumboro disease (Infectious bursal disease), mainly characterized by severe changes in the bursa of Fabricius followed by immunosuppression (**Islam *et al.*, 2012**). IBD is caused by Infectious bursal disease virus (IBDV), a double-stranded RNA Avibirnavirus (**Ferrero *et al.*, 2015**). Moreover, IBDV is extremely contagious, a self-limiting disease and causes mortality of young chicks of both, domestic (chickens and turkeys) and wild birds (guinea fowl, quail, ducks, and pheasants) (**Daodu *et al.*, 2018**).

However, for the control of this infectious viral diseases of poultry, vaccination strategies are essentials. At present, live attenuated, killed, immune complex, and vector vaccines of IBD are commercially available (**Eterradossi and Saif, 2020**). Moreover, live attenuated vaccines are categorized into Mild, intermediate, and intermediate-plus or hot vaccines (**Olesen *et al.*, 2018**). In contrast with mild vaccines, the intermediate and intermediate-plus vaccines give better immunity against IBDV. Although "intermediate" and "intermediate plus" or "hot" vaccines are much more effective and may overcome greater levels of maternally derived antibodies (MDA), but they can also result in moderate to serious bursal lesions and, as a result, cause appropriate concentration of immunosuppression (**Müller *et al.*, 2012**). The MDA are those antibodies which are transferred from mother to offspring's and protect neonates and newborns during the time of their maturation of immune system. The massive common of maternal antibodies are of the IgG isotype (**Niewiesk, 2014**). Consequently, the efficient of a vaccine, depends on the time of vaccination, which can be affected by residual MDA levels (**Jackwood, 2017**). Flocks are IBD-vaccinated between 1 day before, at, or up to 3 days after the estimated optimal time point because, in this period the humoral immunity will be developed and detectable which remain up to 14 days of post vaccination (**Block *et al.*, 2007**). Basically, the optimal vaccination time depends upon the MDA level of the chicks (**Block *et al.*, 2007**). Because, the high titer of maternal antibodies interferes with the multiplication of live vaccine's virus and diminish the level of immunity that could be produced in the chicks. The application of live vaccines during the 1st week of hatch in chicks against diseases whose MDA still persist in the body of the chick will result in defusing of antigen and active immunity may not be delivered by the vaccine (**Pitcovski *et al.*, 2003**). Different serological methods are available to detect the maternal antibody and the antibody provided by the vaccine. Among the different methods, enzyme linked immunosorbent assay (ELISA) is used most commonly as it is sensitive, specific and quantitative. Commercial ELISA kits are available to detect antibodies to IBDV from sera samples (**Martinez-Torrecuadrada *et al.*, 2000**; **Wang *et al.*, 2008**). Though the several studies on the detection of IBDV antibodies were performed in Bangladesh (**Khan *et al.*, 2009**; **Meher *et al.*, 2017**), but limited number of studies on the detection of MDA for IBDV and screening of antibody titer developed after the vaccination. Additionally, the real-time information of humoral response to vaccination is essential to develop and incorporate the mapping tools for veterinary services to control and prevent IBD (**García *et al.*, 2021**). Hence, this study was designed to determine the MDA titer and compare the two commercially available vaccines strains of IBDV (one is "GM-97 strain Intermediate plus" and another one is "Intermediate type strain") in terms of antibody titer in layer chickens.

MATERIALS AND METHODS

Ethical approval

The study was performed in line with the research ethics and strategies as well as the animal care followed by the Department of Microbiology and Public Health, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh. Therefore,

the memo number is BSMRAU/FVMAS/MPH/20(Ethical Approval)/2020/02, Date: 31-01-2021.

Study design, sample collection, transportation and processing

In this study, a total of 1500 layer birds that was originated from Novogen Brown were collected and equally allocated into three groups (group-A, Group-B and Control). All the birds were apparently healthy and reared in standard housing condition as well as the management was same for young layer birds. Two commercially available two different IBD vaccines, "GM-97 strain of Intermediate plus" and "Intermediate type" was used to vaccinate the group-A and Group-B respectively. The third group was control and remained unvaccinated. The birds were also vaccinated by following the recommended procedures of the respective vaccine manufacturing companies. However, all the birds were originated from IBD vaccinated breeders. Birds were fed ad libitum commercial diet and raised under measured states based on the regulations of national animal welfare. The birds were vaccinated by IBD vaccines at 7 days of age and subsequently were re-vaccinated at 18 days of age to boost up the immunity. Blood samples of 16 in number in each time were collected randomly (without grouping into A, B and control) from layer birds at the age of 1 day and 7 days (immediately before vaccination) to determine the maternally derived antibodies (MDA). Then the blood samples were collected from layer birds of all the groups (group-A, group-B and control) at the age of 25 days old (1 week after re-vaccination), 32 days old (2 week after re-vaccination), and 39 days old (3 week after re-vaccination) to estimate the antibody titer. The samples were 16 in number from each group in each time. The blood samples were collected from the large vein under the wing (brachial vein) of live birds with considering the animal welfare policy. Blood samples were collected without anticoagulant to obtain the serum or antiserum. Immediately after collection, the blood samples were sent to the Sufian Agro Care Lab, Birujuli, Kapasia, Gazipur in ice box with ice for serological test. All the serum samples were obtained by processing according to the methods followed by **Meher *et al.* (2021)** to determine the antibody titer by ELISA. In brief, after coagulation of blood, the serum was subjected to spin at 3000 rpm for 5 min to remove the remaining clots, red blood cells, and other insoluble materials. Finally, stored at −20°C for performing the indirect ELISA. In addition, four-layer birds from each group (group-A, group-B and control), a total of 12 were randomly selected at the age of 25 days (1 week after re-vaccination). Similarly, 12 birds at the of 32 days (2 week after re-vaccination) and 12 birds at the of 39 (3 week after re-vaccination) days old. Then the birds were weighed and sacrificed to determine the body weight (BW), Bursa of Fabricius weight (BF) and histopathological lesion score (HLS).

Detection of pre and post vaccinated antibody titer by serological test (indirect ELISA)

The researcher assessed the serum samples by indirect enzyme-linked immunosorbent assay test (iELISA) to determine the antibody titer. It is a quantitative test for the detection of specific antibodies from serum samples. The commercially available ELISA test kit (ID Screen® IBDV Indirect, ID. Vet, Grabels, France) containing IBDV antigen-coated plates were used to measure the antibody titers. This study strictly followed the manufacturer's instructions to perform the iELISA test. Briefly, the protocol suggested to dilute the serum samples at the ratio of 1:50 in dilution buffer, followed by 1:10 dilution. Finally, 1:500 dilution was prepared and used as working sample for iELISA. Then, 100 µl of negative and positive controls were added into A1, B1 and C1, D1 wells of antigen coated plate respectively. Remaining 92 wells were filled with 100 µl of diluted serum samples and the plate was incubated for 30 min at 21°C (±5°C) in dark condition. Meanwhile, according to manufacturer's instructions, the conjugate and wash solutions were prepared. After incubation, each well was aspirated and washed 3 times with approximately 300 µl of the wash solution. The wells should be avoided to dry between the washes. Then, 100 µl of the prepared conjugate was added into each well and incubated for 30 min at 21°C (±5°C). After that, the plate was washed with wash buffer as previously did in the above. Then, each well of microtiter plate was filled with 100 µl substrate solutions and kept at 21˚C (± 5˚C) for 15 min ± 2 min. After incubation, 100 µl stop solutions was added to stop the reaction. Finally, the optical density value of each sample was measured at 405 nm within 15 min after adding stop solution, and recorded by calculating sample to positive (S/P) ratio and antibody titer. The result was validated based on the manufacturer's recommendation that the mean OD (Optical Density) value of the Positive Control (OD PC) must be greater than 0.250, and the ratio of the mean values of the positive and negative Controls (ODPC and ODNC) must be greater than 3.

Calculation of results

For each sample, S/P ratio and antibody titer were calculated using the following formulas:

$$\mathbf{S/P} = \frac{\text{OD of sample} - \text{OD of negative control}}{\text{OD of positive control} - \text{OD of negative control}}$$

Antibody titer for IBVr: $\text{Log}_{10}(\text{titer}) = 1.0 \times \log_{10}(\text{S/P}) + 3.63$;
$\text{Titer} = 10^{\log_{10}(\text{titer})}$

Interpretation of results

S/P Value	ELISA Antibody Titer	IBD Immune Status
S/P ≤ 0.2	Titer ≤ 853	Negative
S/P > 0.2	Titer > 853	Positive

Bursa of Fabricius: body weight (BF:BW) ratio, BF:BW index and histopathological lesions score (HLS)

The BF:BW ratio and BF:BW index were calculated by the following formula,

$$BF{:}\,BW\ ratio = \frac{\text{Bursa of Fabricius weight (gm)}}{\text{Body Weight (gm)}} \times 1000$$

$$BF{:}\,BW\ index = \frac{\text{BF: BW ratio of vaccinated birds}}{\text{BF: BW ratio of un vaccinated birds (control group)}}$$

HLS of Bursa of Fabricius was calculated according to the methods of **Muskett *et al.* (1979)** using the following scale: (0) No damage; (1) mild necrosis in isolated follicles; (2) moderate generalized lymphocyte depletion or isolated follicles, with severe depletion; (3) over 50% of follicles with severe lymphocyte depletion; (4) outline of follicles only remaining with few lymphocytes and increase in connective tissue, cysts, and thickened corrugated epithelium; and (5) loss of all follicular architecture with fibroplasia. All the tissue sections of Bursa of Fabricius were prepared for histopathological observation according to the methods followed by **Afrin *et al.* (2021)**.

Statistical analysis

Data were entered into SPSS version 25 to perform the statistical test. Thus, data were compared within the Group-A, Group-B and Control by performing the One-way Analysis of Variance (ANOVA) and followed by post-hock test (Student-Newman-Keuls test). The antibody mean titer within the groups was compared by Repeated measure Analysis of Variance (ANOVA). Bonferroni test was applied to assess the mean effect among the different ages. All the individual samples of group-A, group-B and control were considered to perform one sample t test to compare the antibody mean titre of each group to the marginal level of protective antibody titre (>853). Before performing the statistical test, all assumption for the specific statistical test were assessed and found too good. The p value <0.05 were assumed to statistically significant. The effect size of one sample t test was measured by using the following formula.

$$\boldsymbol{Effect\ size} = \frac{\text{t}}{\sqrt{N}}$$

Here, N= Sample size and
t= t value of one sample t test

RESULTS

In this study, the antibody titers of layer birds were detected by the iELISA. Both, the group A and B showed, a significantly ($p<0.01$) upward tendency of antibody mean titer and the control group had a significantly ($p<0.01$) descending trend according to their age (Table 1). Surprisingly, the antibody mean titer of both groups (vaccinated) were significantly ($p<0.01$) increased than the MDA mean titer. The highest MDA mean titer of IBD was 6227.69±327.63 observed at the age of 1 days and then gradually decreased to 2075.50±215.22 at the age of 7 days. After vaccination, both vaccinated groups had an upward trend of antibody mean titer and continued up to the age of 39 days (Figure 1). It is very clear from the Figure 1 that the group vaccinated by "GM-97 strain of Intermediate plus" (group-A) had higher trend of antibody mean titers than the group vaccinated by "Intermediate type" (group- B) for all estimated ages layer birds. The line graph of antibody means titers of control group had the tendency to go down and below the protective level. The Table 1 also shows the significant difference between the groups for all estimated ages of layer birds, where the group-A birds had significantly ($p<0.01$) higher antibody mean titer than group-B and control. The highest antibody mean titer was 9121.94±657.05 at the age of 39 days in group-A layer birds. And the lowest antibody mean titer was 469.38±80.7 in control group at the age of 39 days. The antibody titer range within the samples of same group, the highest (10219) was observed at the age of 39 days in group-A and the lowest (1019) in control group (Table 2).

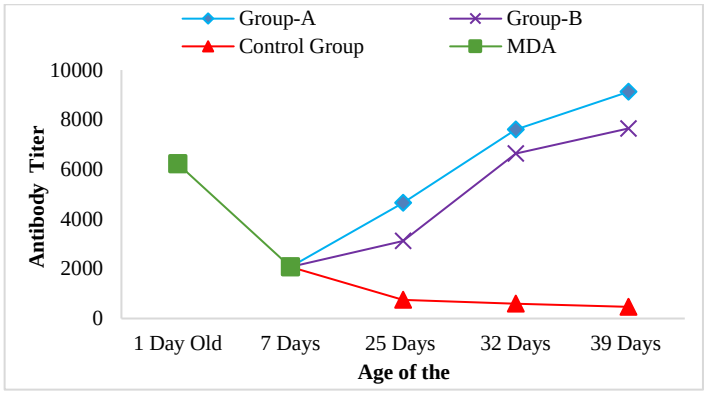

Figure 1 Antibody titer against Infectious Bursal Diseases (IBD) in vaccinated group and Control group at different ages of Layer Birds. 1 day old= MDA titer at 1 Day, 7 days = MDA titer at 7 days old, 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination

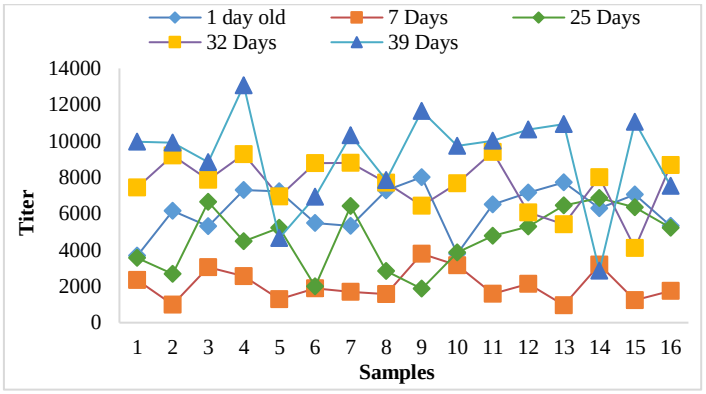

Figure 2 Antibody titer against Infectious Bursal Diseases (IBD) at different ages of Layer Birds in Group-A. 1 day old= MDA titer at 1 Day, 7 days = MDA titer at 7 days old, 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination

Table 1 Infectious Bursal Diseases vaccine antibody titer (Mean ± SEM) at different ages birds of Group-A, Group-B and Control

Group level	Antibody titer (Mean ± SEM)					F value	P value	LS
	Age of the Birds							
	MDA Titer		Vaccinated Titer					
	1 Day Old	7 Days	25 Days	32 Days	39 Days			
Group -A	6227.69^{wx}±327.63	2075.50^{y}±215.22	4657.38^{ax}±423.52	7608.94^{avw}±375.87	9121.94^{av}±657.05	37.56	<0.001	**
Group -B	6227.69^{v}±327.63	2075.50^{w}±215.22	3120.94^{bw}±351.34	6635.81^{bv}±431.99	7648.75^{bv}±511.58	45.16	<0.001	**
Control	6227.69^{v}±327.63	2075.50^{w}±215.22	752.44^{cx}±170.96	595.94^{cx}±80.14	469.38^{cx}±80.7	155.65	<0.001	**
F value			34.97	129.53	91.85			
P value			<0.001	<0.001	<0.001			
Level of significance			**	**	**			

$^{a, b, c:}$ Row values with same letters do not differ significantly; $^{v, w, x, y, z:}$ Row values with same letters do not differ significantly; ** Level of significance at 1% (p<0.01), NS: Insignificant; LS = Level of significance; SEM: Standard Error of Mean; 1 day old= MDA titer at 1 Day, 7 days = MDA titer at 7 days old, 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination.

Table 2 Infectious Bursal Diseases vaccine antibody titer range (Maximum - Minimum) at different age's birds of Group-A, Group-B and Control

Group level	Antibody titer range (Minimum - Maximum)				
	Age of the Birds				
	1 Day Old	7 Days	25 Days	32 Days	39 Days
Group –A			4998(1864-6862)	5299(4103-9402)	10219(2855-13074)
Group -B	4334(3679-8013)	2830(963-3793)	5427(1248-6675)	6076(3569-9645)	7479(3179-10658)
Control			2128(128-2256)	1076(145-1221)	1019(109-1128)

1 day old= MDA titer at 1 Day, 7 days = MDA titer at 7 days old, 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination.

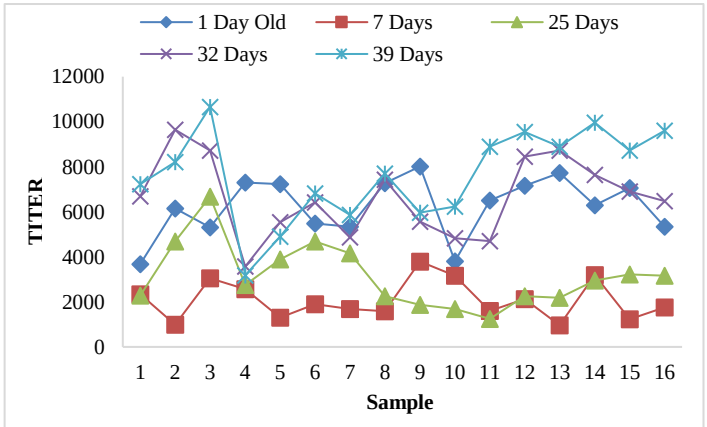

Figure 3 Antibody titer against Infectious Bursal Diseases (IBD) at different ages of Layer Birds in Group-B. 1 day old= MDA titer at 1 Day, 7 days = MDA titer at 7 days old, 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination

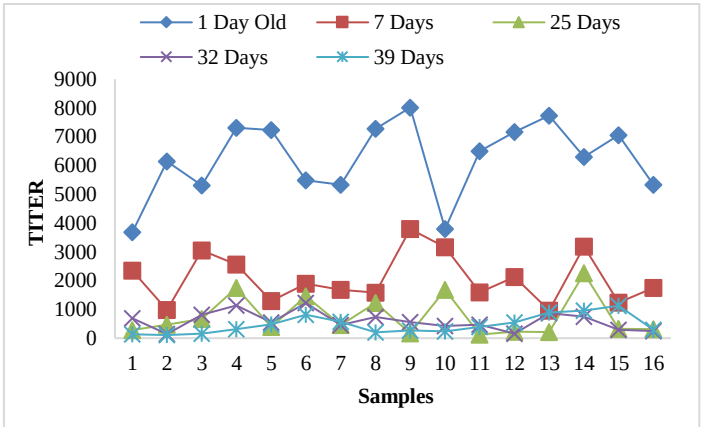

Figure 4 Antibody titer against Infectious Bursal Diseases (IBD) at different ages of Layer Birds in Control Group. 1 day old= MDA titer at 1 Day, 7 days = MDA titer at 7 days old, 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination

The individual samples antibody titer is presented in the Figure 2, 3 and 4 for the birds of Group-A, Group-B and Control respectively. The IBD antibody titer of control group's birds showed that their titer at 25 days fluctuated rapidly within the samples than the antibody titer of intermediate and intermediate plus strain vaccinated groups at different ages. The MDA titer also fluctuated markedly with in the samples at the age of 1 days. The table 3 shows that the antibody mean titer and MDA mean titer were significantly (p<0.01) higher than the protective titer

(>853) for both Group-A and Group-B. On the other hand, the antibody mean titer of control group showed significantly lower than the protective titer (>853) at the age of 32 and 39 days.

Table 3 Comparison of mean antibody titer of each age of different groups birds with the positive antibody titer (>853)

Test Value: > 853							
Variable		t value	Effect Size	*P value (2-tailed)*	Mean Difference	95% Confidence Interval	
Category	Level					Lower	Upper
MDA	1 Day Old	16.40	4.10	0.00	5373.69	4675.36	6072.01
	7 Days	5.68	1.42	0.00	1221.50	762.79	1680.22
Group-A	25 Days	8.98	2.25	0.00	3803.38	2900.67	4706.08
	32 Days	17.97	4.49	0.00	6754.94	5953.80	7556.08
	39 Days	12.58	3.15	0.00	8267.94	6867.46	9668.41
Group-B	25 Days	6.45	1.61	0.00	2266.94	1518.07	3015.80
	32 Days	13.38	3.35	0.00	5781.81	4861.04	6702.59
	39 Days	13.28	3.32	0.00	6794.75	5704.35	7885.15
Control	25 Days	-0.59	-0.15	0.56	-101.56	-465.95	262.83
	32 Days	-3.22	-0.81	0.01	-258.06	-428.88	-87.25
	39 Days	-4.77	-1.19	0.00	-384.63	-556.64	-212.61

1 day old= MDA titer at 1 Day, 7 days = MDA titer at 7 days old, 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination.

The MDA titer at 1 days of age had the higher effect size (4.10; CI: 4675.36-6072.01; n=16) and mean differences (5373.69) was than the 7 days. In case of group-A, the highest effect size (4.49, CI: 5953.80-7556.08; n=16) was at the age of 32 days and the highest mean differences (8267.94; CI: 6867.46-9668.41; n=16) was at the 39 days old birds. The layer birds of Group-B had the highest effect size (3.35; CI: 4861.04-6702.59; n=16) was at the age of 32 days. Interestingly, the birds of control group had the negative effect size at all the ages except their MDA. The bursa of Fabricius weight was increased according to the age of the birds of each group (Table 4).

Table 4 The Bursa of Fabricius weight (Mean±SEM) at different age's birds of different Groups

Group level	Bursa of Fabricius weight (Mean±SEM)			F value	P value	LS
	25 Days	32 Days	39 Days			
Group -A	0.95^{ay}±0.06	1.45^{ax}±0.11	1.95^{ax}±0.12	24.57	0.005	**
Group -B	0.79^{bx}±0.03	1.35^{ay}±0.05	1.77^{az}±0.04	94.86	0.000	**
Control	0.63^{cz}±0.04	1.25^{ay}±0.03	1.68^{ax}±0.06	121.28	0.001	
F value	12.83	1.77	2.64			
P value	0.002	2.25	1.25			
LS	**	NS	NS			

[a, b, c:] Column values with same letters do not differ significantly; [x, y, z:] Row values with same letters do not differ significantly; ** Level of significance at 1% (p<0.01), NS: Insignificant; LS = Level of significance; SEM: Standard Error of Mean; 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination.

The highest bursa mean weight was 1.95±0.12 found at the age 39 days in group-A birds vaccinated by GM-97 strain of Intermediate plus, but there was no significant difference with the control and group vaccinated by "Intermediate type" (Group- B). Only the significant difference was observed at the age of 25 days. None of the vaccines hamper the growth of birds' which was reflected by the significantly (p<0.01) increased body weight according to their age. Even though, the highest growth rate was observed in birds vaccinated by GM-97 strain of Intermediate plus (group A) where the body weight was 534.5±18.98 at the age of 39 days (Table 5). BF:BW ratios at 25 days of age were significantly (p<0.01) higher (7.53±0.28) in group A than others (Table 6). But in the other ages there was no significant difference among the groups in terms of the BF:BW ratios. Though the BF:BW index was highest (1.53±0.09) in group-A at 25 days, but at 39 days old the mean of BF:BW index was higher in group-B. There was no any significant (p>0.05) difference between the vaccinated group in contrast of BF:BW index.

Table 5 The Live Body weight weight (Mean±SEM) at different age's birds of different Groups

Group level	Live Body weight (Mean±SEM)			F value	P value	LS
	25 Days	32 Days	39 Days			
Group -A	126.25^{az}±4.64	210.25^{ay}±3.2	534.5^{ax}±18.98	423.72	0.00	**
Group -B	121.5^{az}±5.85	181.75^{by}±4.4	492.25^{bx}±9.06	1314.25	0.00	**
Control	127.5^{az}±4.73	192.25^{by}±5.98	472.5^{bx}±4.17	1739.91	0.00	**
F value	0.38	9.54	6.54			
P value	0.692	0.006	0.18			
LS	NS	**	*			

[a, b, c:] Column values with same letters do not differ significantly; [x, y, z:] Row values with same letters do not differ significantly; ** Level of significance at 1% (p<0.01), NS: Insignificant; LS = Level of significance; SEM: Standard Error of Mean; 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination.

Table 6 The Bursa of Fabricius: Live Body weight ratio and Index (Mean±SEM) at different age's birds of different Groups

Group level	Ratio (Mean±SEM)			Index (Mean±SEM)		
	25 Days	32 Days	39 Days	25 Days	32 Days	39 Days
Group -A	7.53^{a}±0.28	6.88^{a}±0.53	3.66^{a}±0.29	1.53±0.09	1.07±0.11	1.03±0.09
Group -B	6.55^{b}±0.27	7.42^{a}±0.22	3.59^{a}±0.09	1.34±0.09	1.15±0.07	1.01±0.01
Control	4.95^{c}±0.29	6.53^{a}±0.33	3.56^{a}±0.11	-	-	-
F/t value	21.47	1.35	0.08	1.55^{t}	0.618^{t}	0.271^{t}
P value	0.00	0.307	0.921	0.175	0.560	0.796
LS	**	NS	NS	NS	NS	NS

[a, b, c:] Column values with same letters do not differ significantly; ** Level of significance at 1% (p<0.01), NS: Insignificant; LS = Level of significance; SEM: Standard Error of Mean; [t]= t value of independent t test; 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination.

The table 7 Shows that the mean of histopathological lesion scores (HLS) was gradually decreases according to increasing their (birds) ages. Interestingly, the mean of HLS was significantly (p<0.01) decreased up to 39 days of old only in the control group (non-vaccinated). At the age of 25 days, the HLS mean of the

vaccinated group was not significantly ($p<0.01$) differed with the control group. But at the age of 32 and 39 days only the HLS mean of group-A significantly ($p<0.01$) differed than the control group and had the highest HLS mean of 4.75±0.25 and 3.5±0.65 respectively.

Table 7 The histopathological lesion scores (HLS) of the bursa of Fabricius at different ages birds of different Groups

Group level	The HLS of the bursa of Fabricius (Mean±SEM)			F value	P value	LS
	25 Days	32 Days	39 Days			
Group -A	4.25^{ax}±0.25	4.75^{ax}±0.25	3.5^{ax}±0.65	1.96	0.248	NS
Group -B	3.5^{ax}±0.65	3.25^{ax}±0.85	2.25^{abx}±0.63	1.0	0.403	NS
Control	2.75^{ax}±0.48	1.5^{by}±0.29	1.25^{bxy}±0.25	10.33	0.49	*
F value	2.38	9.07	4.38			
P value	0.148	0.007	0.047			
LS	NS	**	*			

$^{a, b, c:}$ Column values with same letters do not differ significantly; $^{x, y, z:}$ Row values with same letters do not differ significantly; ** Level of significance at 1% ($p<0.01$), NS: Insignificant; LS = Level of significance; SEM: Standard Error of Mean; 25 days= 7 days after vaccination, 32 Days = 14 days after vaccination and 39 Days = 21 days after vaccination.

DISCUSSION

In this study, both the vaccines are capable to produce protective immunity increase at 39 days (3 w.p.v.), which was reflected by comparing with the non-vaccinated group (control). Similar observation also reported by other study where the IBD live-vaccinated birds showed a significant IBDV antibody titer increase at 42 days and the non-vaccinated group gradually decreased (**Prandini *et al.*, 2016**). The author **Prandini *et al.* (2016)** also reported that the IBD-neutralizing antibody titer significantly ($p <0.05$) higher in the groups of bird that vaccinated by intermediate and the intermediate plus vaccines of IBD compared to the non-vaccinated group which is in line with the present study.

The maternally derived antibody (MDA) was in protective level with an increased amount. Specially, the offspring of the vaccinated breeders would have high titers of passive immunity just after hatching (**Michell *et al.*, 2009**). This immunity (MDA) may remain in protective level up to 7 days of post hatching. some reports revealed that the passively immunized chicks (MDA) when vaccinated with an intermediate IBDV strain in the first day of age did not show an increase in antibody titers (**Moraes *et al.*, 2005**). The authors **Thomrongsuwannakij *et al.* (2021)** suggested that the MDA of IBD had a downward tendency after the hatching and sharply decline to non-protective level after 1 weeks of age. This report has the similarities with the finding of the present study.

This might be due to the 1st dose of vaccine was administered at the 7 days when the MDA start to go down the protective level. Because the high MDA at the time of IBDV vaccination may interfere with the vaccine response and neutralize the vaccine virus under laboratory conditions (**Alam *et al.*, 2002**; **Hair-Bejo *et al.*, 2004**; **Moraes *et al.*, 2005**). In this study, the revaccination was done, Because the flocks vaccinated by 1st dose of IBD vaccine at the optimal time point, can develop the detectable humoral immunity and remain up to 14 days post vaccination (**Block *et al.*, 2007**). Among the two vaccines of IBD ("GM-97 strain of Intermediate plus" and "Intermediate type"), the Intermediate plus type increased the ELISA antibody titer within very short time than the Intermediate type. Because the Intermediate plus types are moderately attenuated whereas the Intermediate type are very attenuated. The authors **Rautenschlein *et al.* (2005)** reported that the Intermediate plus vaccine induced the ELISA antibody levels at 14 days of postvaccination (PV) whereas the intermediate type vaccines induced at 28 days of PV. For this reason, the current study found that the intermediate plus vaccinate birds showed the higher immunity than the intermediate type vaccinated birds. However, another author **Thomrongsuwannakij *et al.* (2021)** reported that an intermediate type (M.B. vaccine) vaccinated broiler birds displayed significantly higher IBD antibody titers than the V217 (intermediate plus vaccine) vaccinated broiler birds. The current study revealed that the highest IBD antibody mean titer was found in the 39 days old vaccinated birds. These findings agree with the other authors (**Jakka *et al.*, 2014**; **Prandini *et al.*, 2016**) who reported that the IBD live-vaccinated birds exhibited a significant IBD antibody titer increase at 42 days of post hatch. Though, the birds of all three groups have significantly increased their live body weight with age, but significant differences were between the groups at 32 and 39 days. The body weight was significantly higher in GM-97 intermediate plus type vaccinated birds. The findings of **Thomrongsuwannakij *et al.* (2021)** oppose to this result, where the authors reported that there were no significant differences between the group of broiler birds. The author **Thomrongsuwannakij *et al.* (2021)** also reported that the bursa weight was higher in vaccinated group up to 29 days old. In the current study, the bursa weight was higher in vaccinated group up to 39 days old. Similarly, the BF: BW ratio was also high in the vaccinated group than the control group, which was also dissimilar with the findings of other author **Thomrongsuwannakij *et al.* (2021)**. This variation might be due the differences in poultry species. The bursa lesions were high in vaccinated group specially vaccinated by intermediate plus type at 25 days and also had the significant differences at 32 and 39 days. The IBD live vaccines may have a significant suppressive effect on B lymphocytes as displayed by histological lesions in bursa of fabrius after vaccination (**Prandini *et al.*, 2016**). However, the bursal lesions may develop later than would be expected from this study in SPF layer-type chickens, due to residual levels of MDA (**McCarty *et al.*, 2005**; **Rautenschlein *et al.*, 2005**). Though, the live vaccine can generate the antibody titer quickly, but the live IBD virus vaccine may be neutralized or break through the increased MDA and induce enduring damage to the young broiler chick's immune response (**Ray *et al.*, 2021**). The factors like vaccine manufacturers guidelines for storage, timing, and due dates, consult veterinarians and health status monitoring before vaccine administration to the birds (**Fesseha, 2020**).

CONCLUSIONS

The results of this study indicate that the protective level of IBDV MDA titer may remain up to 1 week of post hatching. Both the intermediate type and intermediate plus (GM97 strain) are able developed the protective antibody and persists for more than 39 days. Comparatively, the Intermediate plus vaccine can generate higher antibody titer than the intermediate type. In addition, the intermediate plus strain induced significantly higher bursal lesions at 14 and 21 d.p.v., significantly higher bursa of Fabricius weight and BF:BW ratios at 7 d.p.v. Compared to the intermediate type vaccinated group. Finally, it needs to be remembered that only one intermediate and one intermediate plus type IBDV vaccine strain was tested in these field studies. Because the vaccine strain may differ in its efficacy and other characteristics from this intermediate and intermediate plus type IBDV vaccine strains. However, the findings of this study can be used to determine the IBD vaccination program for layer pullets in flocks where IBD live vaccines are applied. Therefore, the further study would be long-term monitoring of antibody titer as well as molecular characterization of vaccine virus strain from bursa of Fabricius after vaccination.

Running (short) title: Comparative Antibody titer of Vaccines of IBDV

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Abbreviations: IBDV= Infectious Bursal Disease Virus, MDA= Maternally Derived Antibody, IBD= Infectious Bursal disease, BW= Body Weight, BF= Bursa of Fabricius , HLS= Histopathological Lesion Score, ELISA= Enzyme Linked Immunosorbent Assay and OD= Optical Density.

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