


Detection of MDA Titer of Infectious Bronchitis Virus and Comparison of Antibody Titer Produced by Two Different Infectious Bronchitis Vaccine

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Abstract

The infectious bronchitis (IB) is divesting infectious diseases of poultry. Hence, this study determined the maternally derived antibody (MDA) titer of IBV and comparison of two IB vaccine in layer. A total of 1600 birds were equally allocated into two groups (group-A and group-B). The group-A and group-B were vaccinated by two different vaccines having the strain "B1-Strain of ND+Massachusetts B-41 Strains of IBV" and "Hitchner-B1 Strain of ND+Massachusetts H120 Strains of IBV" respectively. Blood samples were collected and antibody titer was detected by indirect ELISA test. Results showed that both groups had the protective level of MDA titer and both the vaccines significantly ($p < 0.01$) increased the antibody mean titer than the MDA. Between the groups, the highest antibody mean titer (11544.43 ± 177.51) was observed in group-B at 5 weeks age and had significant ($p < 0.01$) difference with group-A (10222.11 ± 96.96). The effect size of the antibody titer was higher in group-B (14.25; CI: 9173.82-9564.39; $n=46$ at 5 weeks birds) than group-A (9.54; CI: 8580.19-9131.68; $n=46$ at one-day-old birds) respectively. Shortly, the MDA mean titer was in protective condition and both the IB vaccinated groups had the significantly ($p < 0.05$) higher antibody mean titer than the protective level of antibody titer (> 853).

Introduction

Poultry farming is the faster growing and important subsector, assists in upgrading the financial condition as well as contribute to human nutrition (Kim et al., 2018). However, it is the matter of regret that there are several factors that hinder the progression in poultry sector. Among them, increased prevalence of disease caused by reemerging pathogens due to naturally recombination of virus and frequent use of live vaccine (Ali et al., 2015). One of the most economical important diseases of poultry is infectious bronchitis virus (IBV) (Uddin et al., 2016). It is highly contagious and causing devastating economic losses to chickens (Bwala et al., 2018). The Infectious bronchitis (IB) is a highly contagious viral disease of the chickens, usually demarcated as an acute, contagious disease of chickens characterized primarily by respiratory signs.

Especially in laying hens, the IB leads to nephritis and affects the reproductive tract, causing poor quality of egg and loss of egg production with misshapen ova (Z. A. Bhuiyan et al., 2018; Ignjatovic and Sapats, 2000). Infectious bronchitis (IB) is caused by Infectious bronchitis virus (IBV), a single-stranded, positive-sense RNA virus of genus *Gammacoronavirus* member of the *Coronaviridae* family and is an enveloped virus with a single-stranded positive-sense RNA genome (Cavanagh, 2007). The active control of this highly contagious disease could be achieved mainly through mass vaccination and strict biosecurity ((Sjaak) de Wit et al., 2011). In spite of regular vaccination, there may be still risk in vaccinated flock, because it does not raise the protection against different serotype and variant strain of this virus (Callison et al., 2006). Though, the

different vaccine strains are currently using, but the conferred defense also depends on different factors related to vaccine type, including vaccination procedures, schedule and also route of administration (Jordan, 2017). The layers are commonly vaccinated at approximately 8 weekly intervals with live attenuated vaccines and with inactivated vaccines after commencement of lay (Ignjatovic and Galli, 1995). The vaccination timing against this disease has particular debates. Basically, the optimal vaccination time depends upon the maternally derived antibody (MDA) level of the chicks (Block et al., 2007). Because, the high maternal antibodies interfere with reproduction of live vaccines and diminish the level of immunity production 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). On the other hand, the humoral immunity induced by early vaccination may not be optimal, possibly because of interfering with MDA, thus remaining the chicks unprotected against the field viruses' strain and supporting immuno-escape as well as recombination (Saiada et al., 2018). Despite this indication, this practice has been observed towards the hatchery administration and also recently in intensive farming (Abdul-Cader et al., 2018; Franzo et al., 2016). Particularly, the traditional methods suggest the interval between subsequent administrations of vaccine for a better immune response, due to let the poultry for recovery of the tracheal epithelium (van Ginkel et al., 2015).

In recent, many countries are practicing the combined application of multiple vaccines at hatchery level to recover this problem (Abdul-Cader et al., 2018; Franzo et al., 2016). Different serological methods are available to detect the maternal antibody and the antibody provided by the vaccine. Among the different serological methods, indirect enzyme linked immunosorbent assay (indirect ELISA) is used most commonly as it is highly sensitive, specific and quantitative. Commercial indirect ELISA kits are available to detect antibodies of IBV from sera samples (Wang et al., 2008; Martinez-Torrecedrada et al., 2000). Though the several studies on the detection of IBV antibodies, were performed in Bangladesh (Khan et al., 2009; Meher et al., 2017), but limited number of studies on the detection of IBV MDA and antibody 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 the contagious diseases (García et al., 2021).

Moreover; regular monitoring of serum antibody from IB vaccinated flocks for IB antibody titres may help to specify the intensity of vaccine response. Hence, this study was designed to determine the MDA titer against IBV, along with the compare of antibody titer developed by two different IB vaccines in layer chickens.

Materials and Methods

Ethical Approval

The study was completed in accordance 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 approval number is BSMRAU/FVMAS/MPH/20(Ethical Approval)/2020/04, Date: 17-02-2021.

Study Design, Sample Collection, Transportation and Processing

In this study, a total of 1600 birds were equally assigned into two groups (group-A and group-B). all the birds were originated from Novogen Brown. The commercially available two vaccines had different strains namely "B1 Strain of ND+ Massachusetts B 41 Strains of IBV" and "Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IBV" were administered to the birds at 21-28 days of age and then every 2-3 months interval. Particularly, the group-A were vaccinated by the "B1 Strain of ND+ Massachusetts B 41 Strains of IBV" and group-B were vaccinated by "Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IBV" respectively. Both the vaccines were administered through the drinking water. All the birds in this study were originated from IB vaccinated breeders. The commercially available feeds were ad libitum to feed the birds. The birds of both groups were raised under measured states based on the regulations of national animal welfare. The birds of both groups were vaccinated at the age of one weeks.

Sample Collection

A total of 368 blood samples were randomly collected from the birds in which 184 blood samples from each group (group-A and group-B). The blood samples of 46 in number were collected in each time, at the age of one day old (before vaccination), 5 weeks, 10 weeks and 15 weeks old birds of each group to estimate the antibody titer. The blood samples were taken from the large vein under the wing (brachial vein) of live birds without anticoagulant to obtain the serum or antiserum. Blood samples were collected with consideration of the animal welfare policy. Then, the blood samples were sent immediately to the Sufian agro care Lab, Birujuli, Kapasia, Gazipur in ice box with ice for serological test. After that, the serum samples were obtained by processing according to the methods followed by another author (Meher et al., 2021). In brief, after clotting of blood, the serum was exposed to spin at

3000 rpm for 5 min to remove the remaining clots, red blood cells, and other insoluble materials. Lastly, stored at -20°C for execution the indirect ELISA.

Detection of Pre and Post Vaccinated Antibody Titer by Serological Test (indirect ELISA)

The antibody titre of serum samples was measured by indirect enzyme-linked immunosorbent assay test (indirect ELISA). It is a numerical test for the recognition of specific antibodies from serum samples. The market available ELISA test kit (ID Screen® IBV Indirect, ID. Vet, Grabels, France) containing IBV antigen-coated plates were used to measure the antibody titers. All this test (indirect ELISA test) was performed according to the manufacturer's instructions. Briefly, at first the serum samples were diluted at 1:50 dilution in dilution buffer, followed by 1:10 dilution, and 1:500 was ultimate dilution, used as working sample for indirect ELISA. Then, the A1, B1 and C1, D1 wells of antigen coated plate were added by 100 μl of negative and positive controls respectively. Remaining 92 wells were filled with 100 μl of diluted serum samples and the plate was allowed to incubate for 30 min at 21°C ($\pm 5^{\circ}\text{C}$) in dark condition. Meanwhile, the conjugate and wash solutions were arranged as per manufacturer's guidelines. After incubation, each well was enunciated and washed 3 times with around 300 μl of the wash solution to avoid drying of the wells between washes. Then, each well of microtiter plate was filled by 100 μl of conjugate and incubated for 30 min at 21°C ($\pm 5^{\circ}\text{C}$). As per previous methods, the plate was washed with wash buffer. After that, 100 μl substrate solutions were added to each well of microtiter plate and kept at 21°C ($\pm 5^{\circ}\text{C}$) for 15 min \pm 2 min. After incubation, 100 μl stop solutions was added to stop the reaction.

Finally, the optical density value of each sample was determined at 405 nm within 15 min after adding stop solution, and noted by assessing sample to positive (S/P) ratio and antibody titer. The result was authorized based on the manufacturer's guideline that "mean OD 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:

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

$$\text{Antibody titer for IBV: } \log_{10}(\text{titer}) = 1.0 \times \log_{10}(S/P) + 3.63;$$

$$\text{Titer} = 10^{\log_{10}(\text{titer})}$$

Interpretation of Results

| S/P Value | ELISA Antibody Titer | IBV Immune Status |
|----------------|----------------------|-------------------|
| S/P \leq 0.2 | Titer \leq 853 | Negative |
| S/P $>$ 0.2 | Titer $>$ 853 | Positive |

Statistical Analysis

Data were inserted into SPSS software version 25 to perform the statistical test. Therefore, data were compared between the group-A and group-B by carrying out the independent t test. The antibody mean titer within the groups were compared by Repeated measure Analysis of Variance (ANOVA) followed by Bonferroni test to assess the mean effect among the different ages of each group. All the separate samples of group-A and group-B 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 execution all the test, all assumption for the specific statistical test were measured and found too good. The p value <0.05 were assumed too statistically significant. The effect size of one sample t test was measured by using the following formula.

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

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

Results

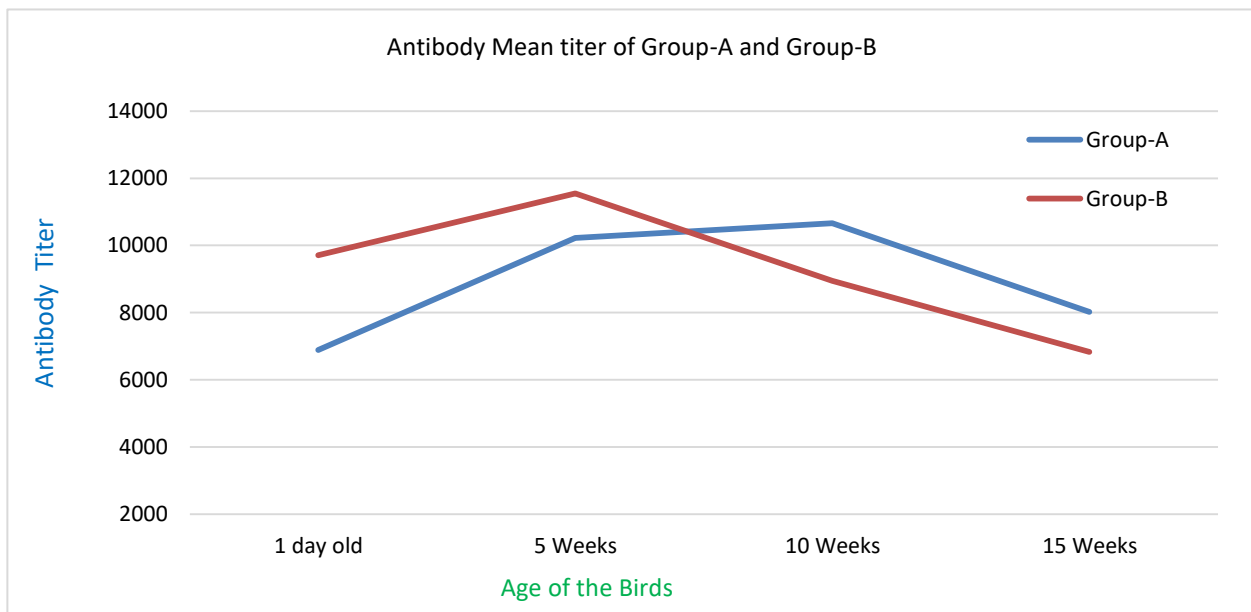
In this study, the antibody titers of layer birds were recorded by the indirect ELISA. Both, the group A and group B showed that the antibody mean titer were significantly ($p < 0.01$) increased and decreased according to their age. Even though, the antibody mean titer (after vaccination) were significantly ($p < 0.01$) increased than the MDA mean titer. Between the two groups, only at the age of 5 weeks had significant differences in the antibody mean titer. The highest antibody mean titer (11544.43 ± 177.51) was observed in Group-B at 5 weeks of age and was significant ($p < 0.01$) difference than the antibody means titer (10222.11 ± 96.96) of Group-B at this stage. The MDA mean titer was higher in Group-B and the antibody titer (just after vaccination) hit the peak and then gradually decreased than the group-A (Figure. 1).

Among the two groups, the lowest antibody titer was MDA, 3054 in Group-A and the highest was 13659 at the age of 5 weeks in Group-B (table 2). The individual samples antibody titer is presented in the Figure 2 and 3 for the birds of Group-A and Group-B respectively.

Table 1. Infectious Bronchitis vaccine antibody titer (Mean±SEM) at different ages birds of Group-A and Group-B.

| Group Level | Antibody titer (Mean ± SEM) | | | | F value | P value | LS |
|-------------|-----------------------------|-------------------------------|-------------------------------|------------------------------|---------|---------|----|
| | Age of the Birds | | | | | | |
| | 1 day old | 5 Weeks | 10 Weeks | 15 Weeks | | | |
| Group-A | 6890.2 ^a ±171.16 | 10222.11 ^b ±96.96 | 10665.89 ^a ±155.53 | 8025.65 ^a ±199.84 | 128.14 | <0.001 | ** |
| Group-B | 9708.93 ^a ±136.9 | 11544.43 ^a ±177.51 | 8950.8 ^a ±194.83 | 6832.24 ^a ±169.13 | 134.45 | <0.001 | ** |
| F value | 0.918 | 13.764 | 3.580 | 1.195 | | | |
| P value | 0.341 | <0.001 | 0.062 | 0.277 | | | |
| LS | NS | ** | NS | NS | | | |

^{a, b, c}: 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.

**Figure 1.** Antibody titer of Infectious Bronchitis (IB) vaccine at different ages of Layer Birds in group-A and group-B.

The IB antibody titer of group-A birds showed that their MDA titer fluctuated rapidly within the samples than the antibody titer of other ages. However, the samples of 5 weeks aged birds showed almost similar trend with little variation. Satisfactorily, all the samples of all ages showed the protective titer (>853), but among them, the MDA titer was lower than the titer of other ages. The highest trend of antibody titer was observed in samples of 10 weeks old birds. The mean titer of different ages birds was significantly higher than the protective antibody titer (>853). The effect size of the antibody titer was higher at 5 weeks aged birds (14.25; CI: 9173.82-9564.39; n=46), but the mean titer was highest at 10 weeks aged bird (9812.89; CI: 9499.64-10126.14, n=46). The antibody titer of group-B birds at 15 weeks of age had more fluctuation with lower trend than the others, even though the MDA titer was higher. In this group (B) the upper trend of antibody titer with in the samples was also observed at 5 weeks of age. Surprisingly, the highest effect size

size (9.54; CI: 8580.19-9131.68; n=46) of antibody titer was recorded at one day old birds (MDA). But the antibody mean titer (10691.43; CI: 10333.91-11048.96, n=46) was higher in the birds of 5 weeks age in Group-B. Like the group-A, the antibody mean titers of all age's birds were also significantly ($p < 0.01$) higher than the protective antibody titer (>853) in the birds of group-B.

Discussion

In this study, all the groups showed that the maternally derived antibody (MDA) were in protective level with an increased amount. These findings are consistent with previous reports of other authors (Michell et al., 2009), who reported that the offspring of the vaccinated breeders would have high titers of passive immunity at hatching. After the vaccination, in group-B the antibody mean titer was increased just after 5 weeks then decline than the MDA.

Table 2. Infectious Bronchitis vaccine antibody titer range (Maximum - Minimum) at different age's birds of Group-A and Group-B.

| Group level | Antibody titer (Maximum - Minimum) | | | |
|-------------|------------------------------------|------------|------------|------------|
| | Age of the Birds | | | |
| | 1 day old | 5 Weeks | 10 Weeks | 15 Weeks |
| Group -A | 3054-9542 | 8864-11620 | 7716-12421 | 4466-10506 |
| Group -B | 6816-11509 | 8578-13659 | 6210-11487 | 4496-8774 |

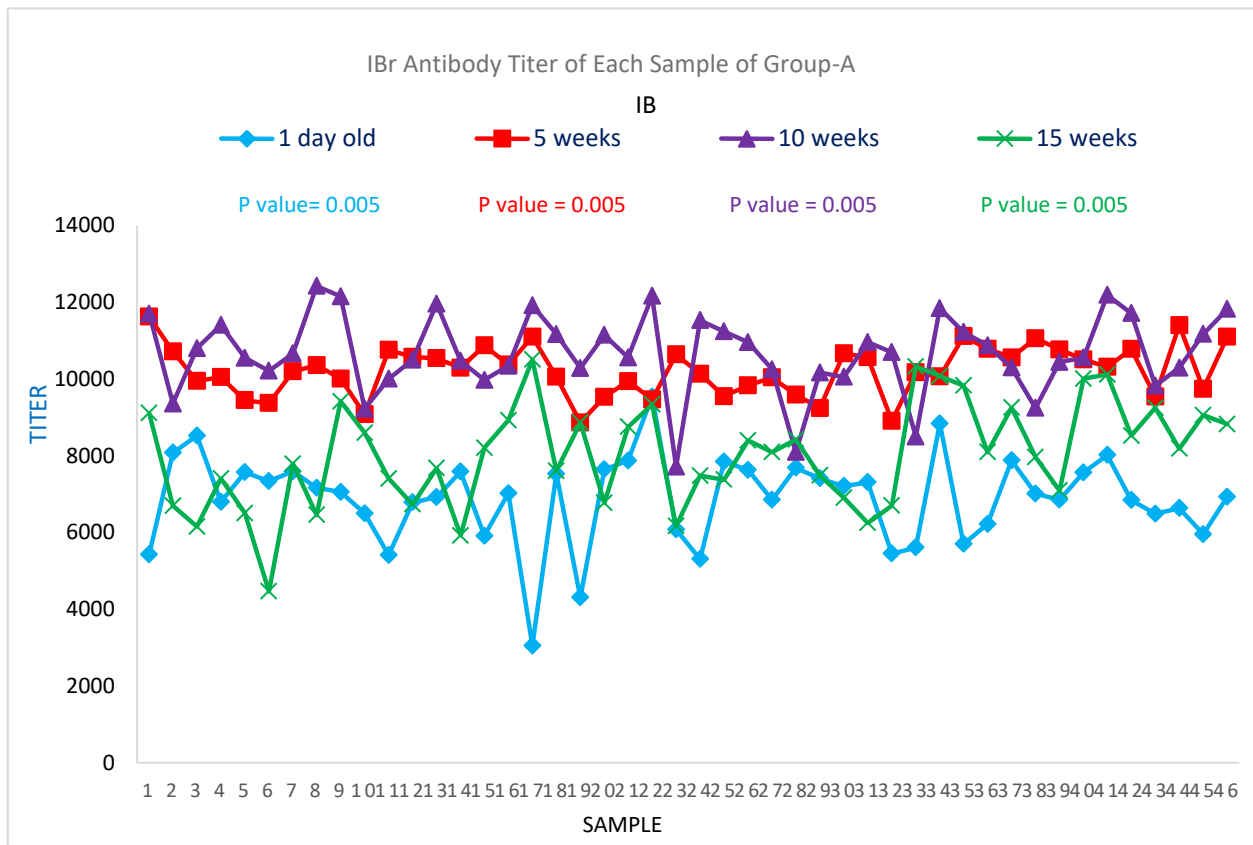


Figure 2. Antibody titer of Infectious Bronchitis (IB) vaccine at different ages of Layer Birds in group-A.

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

| Variable | | Test Value = > 853 | | | | | |
|----------|-----------|--------------------|-------------|--------------------|-----------------|-------------------------|----------|
| Category | Level | t value | Effect Size | P value (2-tailed) | Mean Difference | 95% Confidence Interval | |
| | | | | | | Lower | Upper |
| Group-A | 1 Day Old | 35.27 | 5.20 | 0.000 | 6037.20 | 5692.45 | 6381.94 |
| | 5 Weeks | 96.63 | 14.25 | 0.000 | 9369.11 | 9173.82 | 9564.39 |
| | 10 Weeks | 63.09 | 9.30 | 0.000 | 9812.89 | 9499.64 | 10126.14 |
| | 15 Weeks | 35.89 | 5.29 | 0.000 | 7172.65 | 6770.15 | 7575.15 |
| Group-B | 1 Day Old | 64.69 | 9.54 | 0.000 | 8855.93 | 8580.19 | 9131.68 |
| | 5 Weeks | 60.23 | 8.88 | 0.000 | 10691.43 | 10333.91 | 11048.96 |
| | 10 Weeks | 41.56 | 6.13 | 0.000 | 8097.80 | 7705.40 | 8490.21 |
| | 15 Weeks | 35.35 | 5.21 | 0.000 | 5979.24 | 5638.60 | 6319.88 |

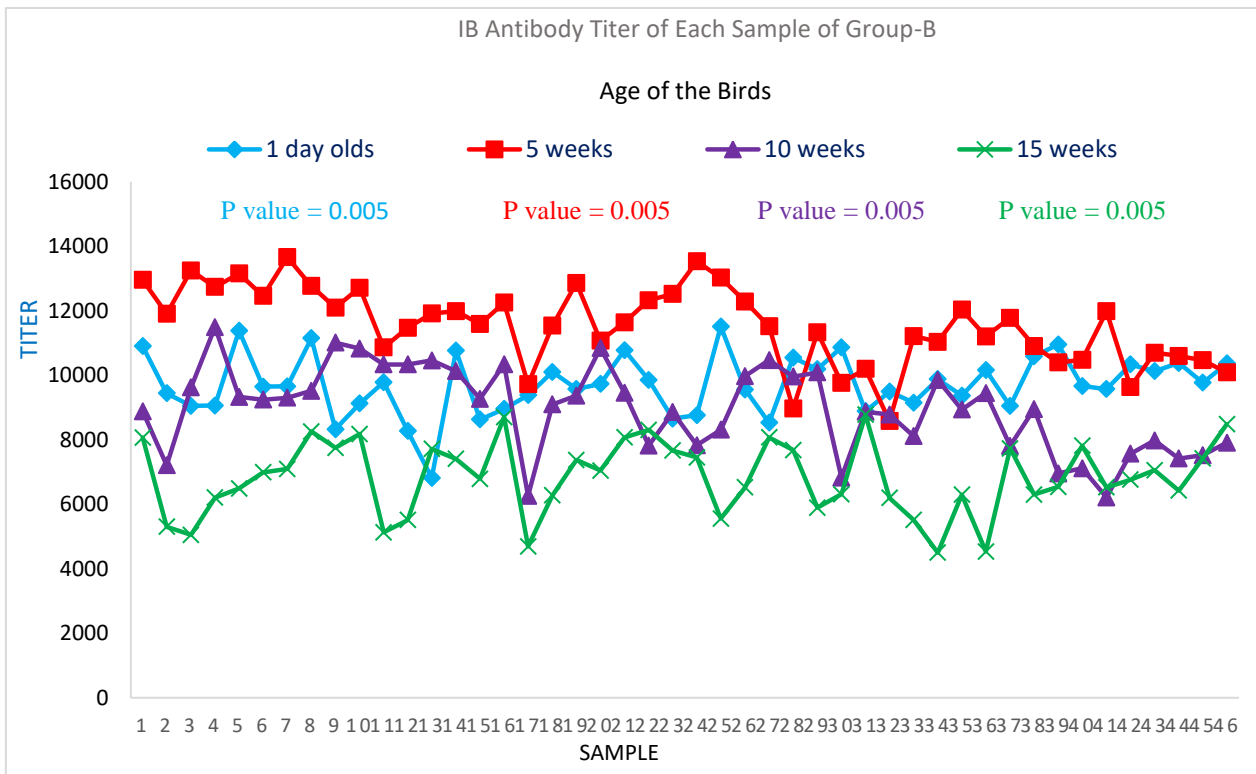


Figure 3. Antibody titer of Infectious Bronchitis (IB) vaccine at different ages of Layer Birds in group-B.

This might be due to the presence of high MDA titer because the authors (Wilson et al., 2014) reported that the MDA-positive vaccinated dogs had decreasing antibody titers following the first vaccination, but surges after the second vaccination. Interestingly, though the birds were originated from same breeder, but the MDA titer was high in group-B. This result suggested by the authors (Coakley et al., 2014) who reported that the transfer of comparative number of antibodies to offspring can vary between females with significantly and consistently. Generally, the MDA of IB remains up to 2 weeks of age, and subsequently, it decreases up to 7 weeks of age (Z. Bhuiyan et al., 2019).

In addition, the increasing trend of IB antibody due to infection is comparatively higher in aged birds especially at 63-73 weeks ages of layer birds (Meher et al., 2017). However, both the group-A and group-B was vaccinated by the Massachusetts serologically related types of Infectious Bronchitis and strains of Newcastle disease virus. These may have resulted the significantly higher IB antibody titer in group-B at 5 weeks, due to secondary effect of suppression of Newcastle disease (ND) virus. The vaccine of ND specially the clone vaccine can produce better immunity (Meher et al., 2021) against the Newcastle disease. The vaccine used in Group-A generate the protective immunity with more stable antibody mean

titer and had the increasing trends up to 10 weeks than the vaccine used in group-B. In this study, the route of administration of the two IB vaccines were same (drinking water method). However, the different vaccine strategies like spray and gel administration having no any significant differences and both are less impactful on body temperature (Legnardi et al., 2021). Therefore, the development of protective immunity within the short time to protect against newly emerging IBV strains could be use of polyvalent vaccines IB vaccine via different vaccination strategies (Shao et al., 2020). Both groups had the significantly higher antibody mean titer than the protective level of antibody titer (>853). These might be due to efficacy of vaccine along with some factors that was practiced regularly. 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

This study revealed that all the freeze-dried live vaccines of IB are able to produce optimum amount of protective antibody titer. Even though, the antibody mean titer (after vaccination) were significantly ($p < 0.01$) increased than the MDA mean titer. The MDA mean titer was in protective condition and did not

hamper the generation of antibody titer after immediately vaccination. Both the IB vaccines had the significantly higher antibody mean titer than the protective level of antibody titer (>853).

Therefore, though the “Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IB” vaccine can produce higher antibody titer than “B1 Strain of ND+ Massachusetts B 41 Strains of IB” vaccines at the beginning, but the antibody titer of “B1 Strain of ND+ Massachusetts B 41 Strains of IB” vaccine decreases slower than “Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IB” vaccine, even though antibody titer was higher for “B1 Strain of ND+ Massachusetts B 41 Strains of IB” vaccine at the last stage. However, the further study could be the compare of different polyvalent IB and IBD vaccines and the detection of antibody titer weekly do determine the effective vaccination regiments.

References

- Abdul-Cader, M. S., Palomino-Tapia, V., Amarasinghe, A., Ahmed-Hassan, H., De Silva Senapathi, U., Abdul-Careem, M. F. (2018). Hatchery Vaccination Against Poultry Viral Diseases: Potential Mechanisms and Limitations. *Viral Immunology*, 31(1), 23–33. <https://doi.org/10.1089/vim.2017.0050>
- Ali, M. Z., Rahman, M. M., Sultana, S. (2015). Seroprevalence of *Mycoplasma gallisepticum* antibody by ELISA and serum plate agglutination test of laying chicken. *Veterinary World*, 8(1), 9. <https://doi.org/10.14202/VETWORLD.2015.9-14>
- Bhuiyan, Z. A., Giasuddin, M., Khan, Z. U. M. (2018). Seroprevalence of infectious bronchitis virus in different types of chicken in Bangladesh. *Asian Journal of Medical and Biological Research*, 4(1), 132–136. <https://doi.org/10.3329/ajmbr.v4i1.36831>
- Bhuiyan, Z., Ali, M., Moula, M., Bary, M., Arefin, N., Giasuddin, M., Khan, Z. (2019). Seroprevalence of major avian respiratory diseases in broiler and sonali chicken in selected areas of Bangladesh. *Journal of Advanced Veterinary and Animal Research*, 6(4), 561. <https://doi.org/10.5455/javar.2019.f383>
<https://doi.org/10.1080/03079450701589175>
- Block, H., Meyer-Block, K., Rebeski, D. E., Scharr, H., de Wit, S., Rohn, K., Rautenschlein, S. (2007). A field study on the significance of vaccination against infectious bursal disease virus (IBDV) at the optimal time point in broiler flocks with maternally derived IBDV antibodies. *Avian Pathology*, 36(5), 401–409.
- Bwala, D. G., Solomon, P., Duncan, N., Wandrag, D. B. R., Abolnik, C. (2018). Assessment of *Mycoplasma gallisepticum* vaccine efficacy in a co-infection challenge model with QX-like infectious bronchitis virus. *Avian Pathology*, 47(3), 261–270. <https://doi.org/10.1080/03079457.2018.1440064>
- Callison, S. A., Hilt, D. A., Boynton, T. O., Sample, B. F., Robison, R., Swayne, D. E., Jackwood, M. W. (2006). Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. *Journal of Virological Methods*, 138(1–2), 60–65. <https://doi.org/10.1016/j.jviromet.2006.07.018>
- Cavanagh, D. (2007). Coronavirus avian infectious bronchitis virus. *Veterinary Research*, 38(2), 281–297. <https://doi.org/10.1051/vetres:2006055>
- Coakley, C. M., Staszewski, V., Herborn, K. A., Cunningham, E. J. (2014). Factors affecting the levels of protection transferred from mother to offspring following immune challenge. *Frontiers in Zoology*, 11(1), 46. <https://doi.org/10.1186/1742-9994-11-46>
- Fesseha, H. (2020). Vaccine Failure in Poultry Production and its Control Methods: A Review. *Biomedical Journal of Scientific Technical Research*, 29(4). <https://doi.org/10.26717/BJSTR.2020.29.004827>
- Franzo, G., Tucciarone, C. M., Blanco, A., Nofrarías, M., Biarnés, M., Cortey, M., Majó, N., Catelli, E., Cecchinato, M. (2016). Effect of different vaccination strategies on IBV QX population dynamics and clinical outbreaks. *Vaccine*, 34(46), 5670–5676. <https://doi.org/10.1016/j.vaccine.2016.09.014>
- García, C., Soriano, J. M., Cortés, V., Sevilla-Navarro, S., Marin, C., Balaguer, J. L., Catalá-Gregori, P. (2021). Monitoring serologic response to single in ovo vaccination with an immune complex vaccine against infectious bursal disease in broilers. *Poultry Science*, 100(4), 100999. <https://doi.org/10.1016/j.psj.2021.01.022>
- Ignjatovic, J., Galli, U. (1995). Immune responses to structural proteins of avian infectious bronchitis virus. *Avian Pathology*, 24(2), 313–332. <https://doi.org/10.1080/03079459508419072>
- Ignjatovic, J., Sapats, S. (2000). Avian infectious bronchitis virus. *Revue Scientifique et Technique de l’OIE*, 19(2), 493–508. <https://doi.org/10.20506/rst.19.2.1228>
- Jordan, B. (2017). Vaccination against infectious bronchitis virus: A continuous challenge. *Veterinary Microbiology*, 206, 137–143. <https://doi.org/10.1016/J.VETMIC.2017.01.002>
- Khan, M., Jahan, S., Paul, M., Chakraborty, D., Islam, M. (2009). Development of an Indirect ELISA technique towards detection of antibodies to Infectious Bursal Disease virus (IBDV) of Chickens. *Bangladesh Vet J*, 43(1–4), 8–16.
- Kim, Y., Biswas, P. K., Giasuddin, M., Hasan, M., Mahmud, R., Chang, Y. M., Essen, S., Samad, M. A., Lewis, N. S., Brown, I. H., Moya, N., Hoque, M. A., Debnath, N. C., Pfeiffer, D. U., Fournié, G. (2018). Prevalence of Avian Influenza A(H5) and A(H9) Viruses in Live Bird Markets, Bangladesh. *Emerging Infectious Diseases*, 24(12), 2309. <https://doi.org/10.3201/EID2412.180879>
- Legnardi, M., Baranyay, H., Simon, C., Molnár, J., Bijlsma, T., Cecchinato, M., Gáspárdy, A., Bersényi, A., Tucciarone, C. M., Franzo, G., & Kőrösi, L. (2021). Infectious Bronchitis Hatchery Vaccination: Comparison between Traditional Spray Administration and a Newly Developed Gel Delivery System in Field Conditions. *Veterinary Sciences*, 8(8), 145. <https://doi.org/10.3390/vetsci8080145>
- Martinez-Torrecuadrada, J. L., Castón, J. R., Castro, M., Carrascosa, J. L., Rodriguez, J. F., & Casal, J. I. (2000). Different Architectures in the Assembly of Infectious Bursal Disease Virus Capsid Proteins Expressed in Insect Cells. *Virology*, 278(2), 322–331. <https://doi.org/10.1006/viro.2000.0559>
- Meher, M. M., Jahan, N., & Afrin, M. (2021). Assessment of Antibody Titer and Lymphoid Organs Weight Following Newcastle Disease Vaccination and Feed-Supplementation of Vitamin-C, Probiotics and Antibiotic-Growth-Promoters in Japanese Quails. *Macedonian Veterinary Review*, 44(2), I–IX. <https://doi.org/10.2478/macvetrev-2021-0016>

- Meher, M. M., Rahman, M., Akter, M. R., & Rahaman, S. (2017). *Detection of Avian Infectious Bronchitis Virus and Its Specific Antibody in Different Ages Layer Birds in Dinajpur District of Bangladesh*. 5(Xi), 2502–2507.
- Michell, B., Gomes, A., Baião, N., Resende, M., Lara, L., & Martins, N. (2009). Effect of maternally-derived antibodies on the performance and immunity of broilers induced by in ovo or post-hatching immunizations with a live vaccine against infectious bursal disease. *Revista Brasileira de Ciência Avícola*, 11(1), 57–63. <https://doi.org/10.1590/S1516-635X2009000100009>
- Pitcovski, J., Gutter, B., Gallili, G., Goldway, M., Perelman, B., Gross, G., Krispel, S., Barbakov, M., & Michael, A. (2003). Development and large-scale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. *Vaccine*, 21(32), 4736–4743. [https://doi.org/10.1016/S0264-410X\(03\)00525-5](https://doi.org/10.1016/S0264-410X(03)00525-5)
- Saiada, F., Eldemery, F., Zegpi, R. A., Gulley, S. L., Mishra, A., Santen, V. L. van, & Toro, H. (2018). Early Vaccination of Chickens Induces Suboptimal Immunity Against Infectious Bronchitis Virus. *Avian Diseases*, 63(1), 38. <https://doi.org/10.1637/11951-081418-Reg.1>
- Shao, G., Chen, T., Feng, K., Zhao, Q., Zhang, X., Li, H., Lin, W., & Xie, Q. (2020). Efficacy of commercial polyvalent avian infectious bronchitis vaccines against Chinese QX-like and TW-like strain via different vaccination strategies. *Poultry Science*, 99(10), 4786–4794. <https://doi.org/10.1016/j.psj.2020.06.062>
- Uddin, M. I., Islam, M. S., Rakib, T. M., Das, S., Kamaruddin, K. M., & Biswas, P. K. (2016). Molecular detection of infectious bronchitis virus isolated from commercial breeder farms in Chittagong District, Bangladesh. *Advances in Animal and Veterinary Sciences*, 4(7), 370–375. <https://doi.org/10.14737/JOURNAL.AAVS/2016/4.7.370.375>
- van Ginkel, F. W., Padgett, J., Martinez-Romero, G., Miller, M. S., Joiner, K. S., & Gulley, S. L. (2015). Age-dependent immune responses and immune protection after avian coronavirus vaccination. *Vaccine*, 33(23), 2655–2661. <https://doi.org/10.1016/j.vaccine.2015.04.026>
- Wang, M.-Y., Hu, H.-L., Suen, S.-Y., Chiu, F.-Y., Shien, J.-H., & Lai, S.-Y. (2008). Development of an enzyme-linked immunosorbent assay for detecting infectious bursal disease virus (IBDV) infection based on the VP3 structural protein. *Veterinary Microbiology*, 131(3–4), 229–236. <https://doi.org/10.1016/j.vetmic.2008.03.010>
- Wilson, S., Siedek, E., Thomas, A., King, V., Stirling, C., Plevová, E., Salt, J., & Sture, G. (2014). Influence of maternally-derived antibodies in 6-week old dogs for the efficacy of a new vaccine to protect dogs against virulent challenge with canine distemper virus, adenovirus or parvovirus. *Trials in Vaccinology*, 3, 107–113. <https://doi.org/10.1016/j.trivac.2014.06.001>