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Day Old Vaccination Against Infectious Bursal Disease in Broiler Chickens

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Abstract: Infectious bursal disease (IBD) also known as Gumboro disease is an important viral disease in poultry industry due to significant economic losses resulting from high mortality and immunosuppression. The disease can only be controlled and prevented by proper vaccination and biosecurity. It was the objective of the study to determine the efficacy of an "intermediate" strain of live attenuated IBD vaccine in broiler chickens at day old vaccination. One hundred and fifty two day-old broiler chicks were reared and raised in slatted cage in experimental house. The chicks were divided into 3 groups namely the groups A, B and C. Both chicks in the group A (day old vaccination) and group B (day fourteen vaccination) were vaccinated with an "intermediate" strain of live attenuated IBD vaccine ($10^{4.5}EID_{50}$/0.1ml) via intraocular route at day old and 14, respectively. The group C acted as the control. Eight chicks from each group A and group C were sacrificed at days 1, 7, 14, 21, 28, 35 and 42. The chicks in the group B were sacrificed at days 14, 21, 28, 35 and 42. The body weight of the sacrificed chicks was recorded and the blood samples were collected for IBD antibody titre using enzyme linked immunosorbent assays (ELISA). On necropsy, the gross pathological changes were recorded. The bursa of Fabricius was fixed in 10% buffered formalin for histological examination. The study showed that the body weight of chickens from all the three groups were not significant different ($p>0.05$) throughout the trial, except at day 14 and day 21 from group A was significantly lowered ($p<0.05$) than group C. The bursa weight and the ratio of bursa weight to body weight showed group B were significantly ($p<0.05$) lowered than group A and group C at days 28, 35 and 42. There were no bursa lesions in group A and group C throughout the experiment. However, moderate bursa lesions was observed in group B at days 21 and 28 of age and showed signs of recovery at days 35 and 42. Single dose of day old vaccination could not induce IBD antibody in the chickens. In contrast, vaccination at day 14 of age induced high and protective level of IBD antibody. The vaccine at both time of vaccination was able to neutralized high level of maternally derived antibody (MDA). It was concluded that single dose of day old vaccination in broiler chickens with high MDA (4821±509) was ineffective and could not induce IBD antibody.

Key words: day old vaccination, "intermediate" strain of live attenuated IBD vaccine, IBD, MDA, ELISA

Introduction

Infectious bursal disease (IBD) also known as Gumboro disease is an acute highly contagious viral infection of 3 to 6 weeks old susceptible chickens (Parkhurst, 1964; Lukert and Saif, 1991). The disease was first reported in USA in 1957 (Cosgrove, 1962) and was relatively under control due to proper vaccination programme both in the hens and chicks. However, in late 1980's outbreak of the disease with high mortality due to very virulent IBD virus (IBDV) was reported in Europe (Chettle *et al.*, 1989) and spread worldwide including in Malaysia in 1991 (Hair-Bejo, 1992, 1993). To date, more than 46 types of imported IBD vaccines are used to control the disease in West Malaysia (Chin, 1993). The vaccines were subjectively classified as the "mild", "intermediate" or "hot" strain IBD vaccines. The "mild" vaccine was found to be unable to neutralize high level of MDA in chickens and failed to induce IBD antibody. In contrast, some of the "intermediate" and most of the "hot" vaccines cause severe bursal lesions as those observed in the field IBD outbreak (Hair-Bejo *et al.*, 2000). The time of vaccination, type of vaccine, maternal derived antibody (MDA) in the chicks and pathogenicity of the IBDV field challenge are important factors determining on the efficacy of IBD vaccination. The MDA is acquired by chicks through the passage of IgG from hen serum to the embryo (Brambell, 1970) with the half-life of about 3-5 days in the chicks. The MDA is known to neutralize IBDV (Wyeth and Cullen, 1976). Thus, it is important to ensure that the level of MDA is high enough to provide protection against IBDV infection (Nunoya *et al.*, 1992), especially during the first 2-3 weeks of age prior to IBD vaccination or otherwise early vaccination might be recommended. In spite of the advantages of day old vaccination which may help in early protection for the chicks against the disease, easy handling, less manpower and also reduce vaccination stress, the efficacy of the vaccination needs further clarification. Day old vaccination in chickens of low or absent MDA using the "intermediate" or "hot" strain of live attenuated IBD vaccine is of high risk of immunosuppression due to severe damages of the bursa of Fabricius. In contrast, the inability of the vaccine virus to fully neutralized MDA could lead to failure of the virus to replicate in the bursa of Fabricus and

unable to induce IBD antibody. It was reported that day old vaccination using oil emulsion IBD vaccine was able to protect at least 85 and 90% of the chicks when challenged with virulent IBDV at 4 and 7 weeks of age, respectively (Wyeth and Chettle, 1990). A combination of commercial live and inactivated vaccination in day old broiler chicks against Newcastle disease (ND) and IBD respectively were effective in preventing clinical disease for ND and IBD for at least 35 days of age (Giambrone and Clay, 1986). Day old vaccination in specific pathogen free (SPF) chickens (Whitfill *et al.*, 1995) and commercial broiler chickens (Haddad *et al.*, 1997) using IBD immune complex vaccine was found to be effective against IBDV infections. It was the objective of the study to determine the efficacy of single dose of day old vaccination in broiler chickens with high MDA using an live attenuated IBD vaccine of "intermediate" strain.

Materials and Methods

Broiler chickens: One hundred and fifty two day-old broiler chicks were reared and raised in slatted cage in experimental house. The chicks were divided into 3 groups namely the groups A, B and C. Both chicks in the group A (day old vaccination) and group B (day fourteen vaccination) were vaccinated with an "intermediate" strain of live attenuated IBD vaccine ($10^{4.5}EID_{50}$/0.1ml) via intraocular route at day old and 14, respectively. The group C acted as the control. Feed and water were given ad libitum. Eight chicks from each group A and group C were sacrificed at days 1, 7, 14, 21, 28, 35 and 42. The chicks in the group B were sacrificed at days 14, 21, 28, 35 and 42. The body weight of the sacrificed chicks was recorded and the blood samples were collected for IBD antibody titre using enzyme linked immunosorbent assays (ELISA). On necropsy, the gross pathological changes were recorded. The bursa of Fabricius was fixed in 10% buffered formalin for histological examination.

Histopathology: The bursal tissues were fixed in 10% buffered formalin. Each tissue was trimmed to the thickness of 5mm in size, fixed and dehydrated in a series of alcohol concentration, and embedded in paraffin wax using an automatic tissue processor. Sectioning of tissue was done to a thickness of 5 micrometer on a microtome. The bursal tissues were mounted on glass slides, dewaxed and stained with Haematoxylin and Eosin (HE). The bursal tissues were examined using x4, x10, and x40 objectives for histological changes. The bursal lesions were subjectively graded as normal (0), mild (1), mild to moderate (2), moderate (3), moderate to severe (4), and severe (5) by a modified scoring method previously established (Hair-Bejo *et al.*, 2000).

IBD antibody titre (ELISA): The ELISA technique was carried out according to the methods described by IDEXX Laboratories Incorporation, USA. Briefly, the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted five hundred folds (1:500) with sample diluent prior to the assay. A 100 µl of diluted sample was then put into each well of the plate. This was followed by 100 µl of undiluted negative control into well A1 and A2, 100 µl of undiluted positive control into well A3 and A4. The plate was incubated for 30 minutes at room temperature. Each well was then washed with approximately 350 µl of distilled water for 3 times. Goat anti-chicken conjugate (100µl) was dispensed into each well. The plate was incubated in room temperature for 30 minutes, followed by washing each well with 350 µl distilled water for 3 times. TMB solution (100 µl) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650nm. IBD antibody titre was calculated automatically, using software by Blankfard and Silk (Blankfard and Silk, 1989).

Statistical analysis: Data was analyzed using 2-way analysis of variance (ANOVA), one-way ANOVA, post hoc test with Duncan and Least Significant Difference (LSD), and student independent t-test by using SPSS v.10.0 for Windows (Norusis, 1999).

Results

Clinical signs: Chickens from all the 3 groups did not exhibit any abnormal clinical signs throughout the experiment.

Gross lesions: No gross lesions were recorded in the chickens from the control group and day old vaccination group throughout the experiment. Gross pathological changes were observed and confined in a few bursa of Fabricius of the chickens in group B at day 21 of age or day 7 post vaccination. The affected bursa was oedematous and covered with yellowish transudate on the mucosal and serosal surfaces of the organ. Some of the bursa also had mild pinpoint necrosis on mucosal surface and others were elongated and atrophied.

Body weight: Body weights of chickens from the control group were significantly increased from day 1 (48.3±0.8g) to day 42 (2166.3±102.6g) throughout the experiment (Fig. 1). The body weights of the chickens from group A and group B also followed the same trend of increment throughout the experiment. There were no statistical differences ($p>0.05$) in body weight between group A and group C throughout the experiment, except at days 14 and 21 where group A was significant lowered ($p<0.05$) in body weight than the group C. There

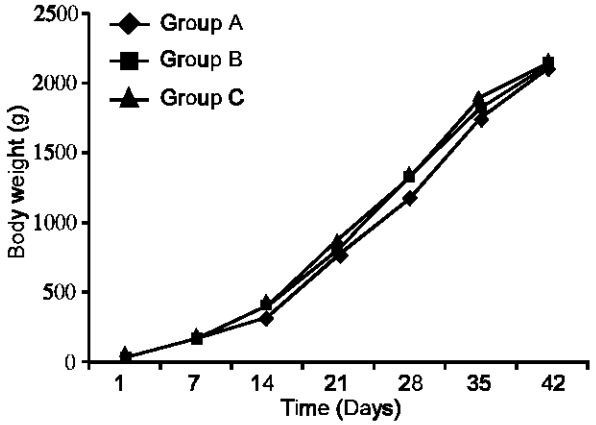

Fig. 1: Body weight of the chickens throughout the experiment

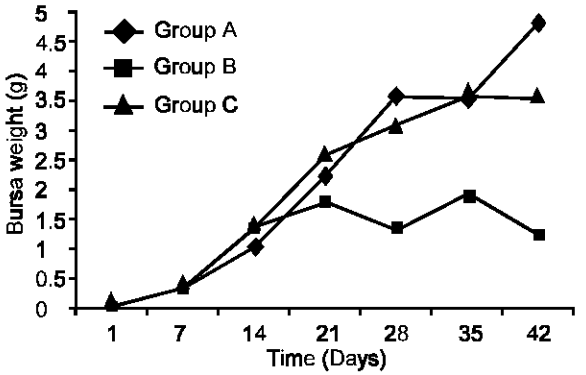

Fig. 2 : Bursa weight of the chickens throughout the experiment

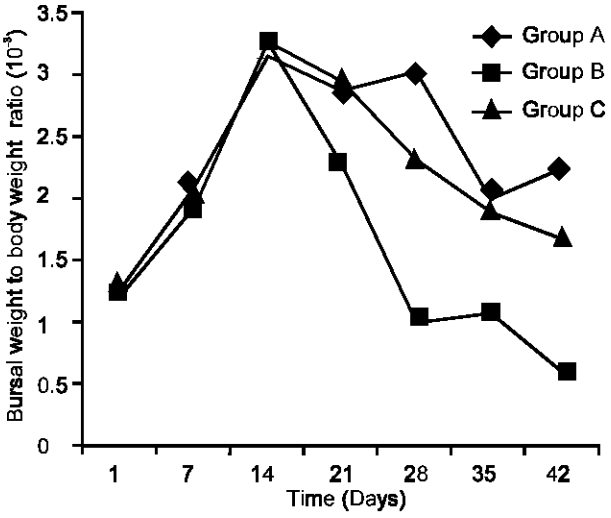

Fig. 3: Bursa to body weight ratio of the chicken throughout the experiment

was no statistical difference (p>0.05) between group B and group C throughout the experiment.

Bursa weight: The interaction between treatment and day was statistically significant (p<0.05). The bursa weight of the chickens in control group was increased from day 1 (0.06±0.00g) to day 35 (3.59±0.34g) and remained high at day 42 (Fig. 2). The bursa weight of the chickens in group A was increased from day 1 (0.06±0.00g) until day 28 (3.56±0.33g) and remained high thereafter. In group B, there was increment of bursa weight from day 1 (0.06±0.00g) to day 21 (1.81±0.32g) as those of the control group and also group A, but it remained low at days 28, 35 and 42. No significance difference (p>0.05) between group A and group C observed throughout the experiment. There were no significance different (p>0.05) between group B and group C throughout the experiment, except at day 28 till day 42, where group B was significantly lowered (p<0.05) in bursa weight of the chickens than group C.

Bursa to body weight ratio ($x10^{-3}$): The interaction between treatment and day was statistically significant (p<0.05). The bursa to body weight ratios were gradually increased (p<0.05) from day 1 (1.23±0.06) to day 14 (3.26±0.14) in control group and decreased on the following days (Fig. 3). The ratio was increased from day 1 (0.06±0.00) to day 14 (3.13±0.48) in group A and decreased from day 14 till day 42. The ratio in group B increased from day 1 to day 14 and decreased thereafter. No significant (p>0.05) difference was observed between group A and group C throughout the experiment, except at day 42, where group A was significantly higher (p<0.05) than group C. At days 28, 35 and 42, group B's ratio was significantly lowered (p<0.05) than group C and group A.

Bursa of Fabricius lesion scoring: The bursa lesions scoring for the control group remained normal to mild throughout the experiment (ranging from 0.00±0.00 to 0.90±0.46) (Fig. 4). The lesion scoring in group A also ranged from normal to mild throughout the experiment (ranging from 0.38±0.11 to 0.90±0.46). As for group B, the lesions ranged from normal to mild from day 1 (0.90±0.46) till day 14 (0.00±0.00), mild to intermediate from day 21 (2.5±0.36) to day 28 (2.75±0.16) and back to mild at day 42 (1.67±0.33).

IBD antibody titre: The interaction between treatment and day was not significant (p>0.05). In control group, the antibody titre was significantly decreased (p<0.05) from day 1 (4821±509) to day 21 (195±54) and remained low thereafter (Fig. 5). The antibody titre of chickens in group A was decreased from day 1 (4821±509) to day 14 (245±105) and remained low at days 21, 28, 35 and 42. The antibody titre in group B decreased from day 1

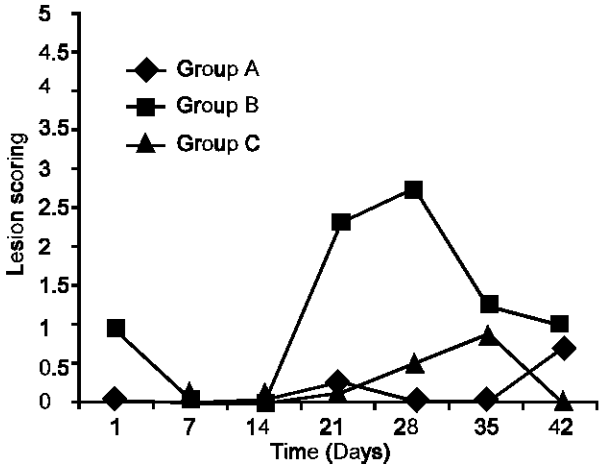

Fig. 4 : Bursa of Fabricius lesions scoring throughout the experiment

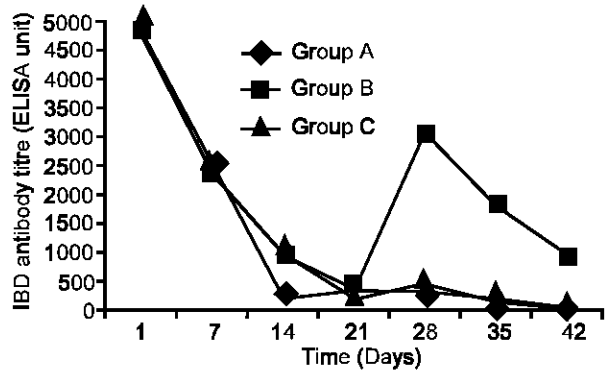

Fig. 5 : IBD antibody titre of the chickens throughout the experiment

(4821±509) to day 21 (365±144) and was significant increased ($p<0.05$) at day 28 (3054±383) and dropped at days 35 (1828±544) and 42 (944±385). There was no significance ($p>0.05$) difference between the IBD antibody titre in group A and group C throughout the experiment except at day 14 where group A was significant lowered ($p<0.05$) than group C. The antibody titre in group B was significant higher ($p<0.05$) than group C from day 28 till day 42.

Discussion

The study showed that day old vaccination can cause reduction in body weight of the chickens at days 14 and 21 of age, although the chickens were recovered at day 28 and able to catch up to market body weight at day 42. The bursa weight, bursa to body weight ratio, gross and histological changes in the day old vaccination chickens were remained normal as those of the control group throughout the trials. In contrast, the bursa weight of the chickens in day 14 vaccination was significant lower ($p<0.05$) than the control group at day 21 and thereafter. This could be due to proliferation of the virus in the bursa of Fabricius and causes atrophy of the organ. The lesion scoring of the bursa ranging from mild to intermediate at day 28 and day 35. However, at day 42, the bursa lesion scoring returned back to mild, which indicates recovering of the organ. It is vital for a vaccine to overcome or neutralize MDA and establish itself in bursa of Fabricius for the induction of high and protective level of IBD antibody.

The IBD antibody titre for day old vaccination is decaying, similar to the antibody titre of the control group, but was significantly lowered at day 14 of age. This suggests that the vaccine virus is being fully neutralized by MDA and thus fail to multiply and proliferate in the bursa of Fabricius and induce IBD antibody. A booster at about 10 to 14 days of age might be useful for the induction of the antibody. In contrast, the day 14 old vaccination showed a significant high and protective level (titre of above 1000 ELISA unit) of IBD antibody titre at day 28 of age and remained protective thereafter. However, booster vaccination might be necessary to be given at day 28 as it could further increased IBD antibody titre at day 35 and thereafter, particularly if the chickens are kept for more than 42 days of age (Hair-Bejo *et al.*, 1998).

It is interesting to find that day old vaccination with live attenuated "intermediate" strain of IBD vaccine in commercial broiler chickens with high MDA (4821±509) was not effective and unable to induce IBD antibody. This is in contrast with the previous report using inactivated (Wyeth and Chettle, 1990; Giambrone and Clay, 1986) or IBD immune complex vaccine (Whitfill *et al.*, 1995; Haddad *et al.*, 1997).

It was concluded that single dose of day old vaccination in broiler chickens with high MDA (4821±509) was ineffective and could not induce IBD antibody. The vaccination at 14 days of age has shown to be effective and able to induce high and protective level of IBD antibody titre up to 42 days of age.

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