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A Comparative Study of Two Vaccines Against Infectious Bursal Disease in Newly Hatched Broiler Chickens

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Abstract: The main objective of this study was to compare two vaccines against infectious bursal disease in broiler chicken with maternally derived antibody. One hundred and sixty eight, 1 day old broiler chickens (ISA 57) were randomly assigned to seven groups, namely; 1, 2 and 3 (V1 (1), V1 (10) V1 (10)) vaccinated Subcutaneously (SC) with D78 IBDV (Intervet) and 4, 5 and 6 V2 (1), V2 (10) V2 (18) vaccinated with transmune vaccine (Ceva) at 1, 10 and 18 days while group 7 were kept as Control (C). All chickens were bled weekly for 49 days post-vaccination for antibody titration using ELISA test. Body weight and thymus, bursa and spleen ratios scores were determined at 21 and 42 days while RNA was extracted from these tissues and run for virus detection studies. The result reveled non-significant differences between groups V1 (1), V1 (10) V2 (1) and V2 (10) in body weight. However, body weight in groups V1 (18) and V2 (18) were significantly different from all other groups at day 21. At 42 days, group V2 (10) was significantly different compared with other groups. However, groups V1 (1), V1 (10) V2 (1) and V2 significantly differed from controls. Antibody titers against NDV were not significantly different in all groups except in the 7th week of the experiment. However, antibody titers against IBDV differed significantly at 4, 5 and 7 weeks post-vaccination in birds vaccinated at day old. Similar results were recorded in 3, 4 and 5 weeks post-vaccination. It is concluded that both vaccines induce an adequate response in performance at 1, 10 and 18 days and both vaccines responded well at the end of the experiment. However, transmune vaccine has been showed significant antibody titers against IBDV in the chickens vaccinated at 18 days old.

Key words: Infectious Bursal Diseases Virus (IBDV), broiler chickens, lymphoid organs, transmune vaccine, RT-PCR

INTRODUCTION

Most countries face problems of poultry meat and egg shortage due to diseases such as Infectious Bursal (IBD) or Gumboro disease. This is one of the most important diseases of commercial poultry farms worldwide (Lukert and Saif, 2003). It is caused by an avibirnavirus resulting in an acute and highly contagious immunosuppressive infection which primarily targets the Bursa of Fabricius (BF); some strains do not protect against the infection in young chicks.

The clinical diseases in older birds causes depression, ruffled feathers, anorexia, white watery diarrhea and dehydration (Chansiripornchai and Sasipreeyajan, 2009). The infection is responsible for high mortality and growth retardation, resulting in significant losses to the industry. IBDV is difficult to eradicate by vaccination, although several types of vaccines have been developed to control it including killed as well as different types of cell culture live attenuated vaccines.

A novel vaccine consisting of specific IBDV antibodies combined with live attenuated IBDV virus strain (2512) has been used to immunize 18 days old embryos *in ovo* or day old chicks Subcutaneously (SC) (Haddad *et al*., 1997; Kelemen *et al*., 2000; Corley *et al*., 2001; Ivan *et al*., 2005). These studies suggested that vaccine administered at day old via SC route induces active immunity despite the presence of variable maternal immunity against IBDV. The vaccine has been used successfully in hatcheries without significant immunosuppressive effect on broiler and SPF eggs.

The objective of this study was to compare the immunogenicity of two commercial live IBDV vaccines given at 1, 10 and 18 days of age by subcutaneous injection under the neck skin and assess their effect on the performance and status of lymphoid organs of broiler chicken.

MATERIALS AND METHODS

Chickens and housing: A total of 168 days old broiler chicks (ISA 57, Entaj Poultry Farms, Saudi Arabia) of comparable weight were randomly assigned to 7 groups: 1-6 and control (group 7). Groups 1-3 were vaccinated

with D78 IBDV vaccine (Nobilis strain D-78 from ((Intervet) at 1, 10 and 18 days old; V1(1), V1(10), V1(18)) while groups 4-6 were vaccinated with transmune vaccine (CEVA-Phylaxia biological company, Budapest, Hungary) at similar days and named: V2 (1), V2 (10), V2 (18). Group 7 kept as Control (C). Each group was divided into four replicates of six chicks each.

Birds were housed in electrically heated battery cages. Lighting was incandescent and continuous throughout the experiment. All birds were fed a commercial starter diet (22% protein and 3100 ME kcal kg^{-1}) to 21 days (starter phase), followed by a finisher diet (18% protein and 3200 ME kcal kg^{-1}) until the termination of the experiment at 42 days of age (22-42 days, finisher phase). Feed and water were provided *ad libitum*. The weights of chickens were recorded weekly.

Tissue collection: Five broiler chickens were randomly taken from each vaccinated group at 21 and 42 days post-vaccination, weighed, blood sampled and euthanized. At necropsy, bursa of fabricius, spleen and thymus were removed from each chicken, weighed and their weight percentages to body weight were calculated before fixing them in 10% formalin.

Vaccination of chicks: Two types of commercial IBDV vaccines were used in this study, nobilis strain D-78 from (Intervet) (V1) and immune complex cevac transmune IBDV vaccine from (CEVA-Phylaxia biological company, Budapest, Hungary), (V2). These were live vaccines and used according to manufacturer's directions and injected subcutaneously. Two groups were vaccinated against ND as booster dose with Lasota strain (Fort Dodge Animal Health-USA) at 21 days old.

Blood sampling: On day 0, half of the chicks of each group were bled (Olorede and Longe, 1999) in order to prepare blood samples of day 0 for chicks of group C (control) and V (eye-drop vaccinated). On day 7, 14, 21, 28, 35 and 42, blood samples were collected from wing veins using 3 mL syringes with 25 gauge needles as described by Zander *et al*. (1997) and Alcorn (2002). In order to minimize biochemical parameters changes, sera were separated and centrifuged at 2300 g for 5 min as recommended by Hrubec *et al*. (2004).

RNA extraction: RNA was extracted from the spleen, bursa and thymus samples fixed in 10% formalin. About 30 mg pieces from each organ were washed with 70% ethanol before RNA extraction. The samples were then disrupted using dry ice and ground using mortar and pestle then homogenized using ultra turrax™ to a homogenous state. Cell lyses was then preformed with the inclusion of β-mercaptoethanol. The illustra™ RNA spin Mini Kit was used to extract RNA from the tissues as per manufacturer recommendation. RNA extraction was quantified using SmartSpect spectrophotometer to access the purity and quantity of the RNA.

Real-time reverse transcriptase PCR amplification: Real-time reverse transcriptase PCR was performed using reagents from Anigen (BioNote recently) IBDV real-time PCR kit from Animal Genetics, Inc. and StepOne™ real-time PCR system was used for amplification. Briefly, the reverse transcriptase was performed at 42°C for 30 min, followed by denaturation and inactivation of reverse transcriptase at 94°C for 15 min. Forty PCR cycles were then performed consisting of denaturation at 94°C for 15 sec and annealing/extension at 60°C for 1 min. In addition, a standard curve was generated using four standard; 1×10^{4} copies uL^{-1}, 1×10^{5} copies uL^{-1}, 1×10^{6} copies uL^{-1}, 1×10^{7} copies uL^{-1}, for quantitative analysis of viral load in the tissue sample. The samples were run in triplicates, two positive and one negative controls as well as the standards to ensure good laboratory practice.

Data analysis for RT-PCR: Data was collected using StepOne™ version 1.0 software that operates on the StepOne™ PCR real-time system (Applied Biosystems). The software allows for PCR reaction to be analyzed as different dyes are used. FAM dye was used as the reporter while ROX was used as the reference dye. Both the threshold cycle and baseline were then set and the settings applied to the assay to generate Ct values for each reaction. The threshold cycle Ct is the PCR cycle number at which the measured florescence signal passes a threshold value after a certain number of cycles. The Ct values were accessed when amplification cure of standard 1-4 passed the threshold line.

Statistical analysis: Data were analyzed using GLM procedure in SAS (1996). The differences were considered significant at level of ($p \leq 0.05$).

RESULTS AND DISCUSSION

Antibody measurement: Antibody titers against NDV in chickens vaccinated at day old with both IBDV vaccines showed no significant differences between all groups except at the 7th week and both groups differed

Table 1: Antibody titration at weekly interval post-vaccination with two IBDV vaccines

		Antibody titration at different weeks							
Groups	Vaccines	0	1	2	3	4	5	6	7
A (1)	NDV	3.82±0.13^{a}	3.73±0.10^{a}	3.42±0.13^{a}	3.04±0.15^{a}	2.72±0.16^{a}	2.91±0.26^{a}	2.96±0.17^{a}	2.52±0.23^{b}
	IBD	2.58±0.28^{a}	2.36±0.11^{a}	1.26±0.36^{a}	0.93±0.39^{a}	1.89±0.44^{b}	2.97±0.20^{b}	3.43±0.13^{a}	3.61±0.08^{a}
B (1)	NDV	3.82±0.13^{a}	3.92±0.10^{a}	3.33±0.12^{a}	2.91±0.18^{a}	3.20±0.21^{a}	3.10±0.01^{a}	3.12±0.34^{a}	3.30±0.18^{a}
	IBD	2.58±0.28^{a}	2.60±0.13^{a}	1.75±0.32^{a}	0.57±0.38^{a}	3.31±0.08^{a}	3.39±0.06^{a}	3.38±0.05^{a}	3.27±0.10^{b}
A (10)	NDV	ND	ND	3.01±0.24^{a}	2.56±0.25^{a}	2.98±0.12^{a}	3.50±0.13^{a}	3.55±0.12^{a}	2.99±0.28^{a}
	IBD	ND	ND	1.14±0.11^{a}	0.12±0.06^{b}	2.53±0.32^{a}	2.90±0.09^{b}	3.37±0.07^{a}	3.35±0.16^{a}
B (10)	NDV	ND	ND	3.10±0.17^{a}	2.74±0.25^{a}	2.96±0.21^{a}	2.89±0.20^{b}	3.32±0.19^{a}	3.07±0.27^{a}
	IBD	ND	ND	1.73±0.11^{a}	0.53±0.23^{a}	1.51±0.08^{b}	3.28±0.08^{a}	3.35±0.06^{a}	3.63±0.06^{a}
A (18)	NDV	ND	ND	ND	2.83±0.14^{a}	3.09±0.05^{a}	3.30±0.16^{a}	3.49±0.13^{a}	3.49±0.06^{a}
	IBD	ND	ND	ND	0.23±0.15^{b}	2.71±0.14^{a}	3.07±0.16^{a}	3.18±0.12^{b}	3.18±0.07^{b}
B (18)	NDV	ND	ND	ND	2.63±0.21^{a}	3.01±0.07^{a}	3.13±0.07^{a}	3.34±0.20^{a}	3.27±0.17^{a}
	IBD	ND	ND	ND	0.13±0.13^{b}	0.78±0.40^{b}	3.30±0.06^{a}	3.40±0.04^{a}	3.66±0.03^{a}
C	NDV	3.82±0.13^{a}	3.05±0.11^{b}	2.55±0.10^{b}	1.84±0.15^{b}	1.12±0.10^{b}	0.67±0.07^{b}	0.50±0.09^{b}	0.31±0.04^{c}
	IBD	2.58±0.28^{a}	2.05±0.13^{b}	1.30±0.11^{ab}	0.90±0.21^{ab}	0.63±0.13^{bc}	0.38±0.02^{bc}	0.28±0.09^{bc}	0.12±0.06^{c}

$^{a-c}$Values with different in the same row with different superscript differ significantly ($p<0.05$)

significantly as compared to control groups. At 10 days vaccinations, there was no significant difference in antibody titers except at the 4th week while vaccination at 18 days with IBDV vaccines revealed no significant differences between the vaccinated groups, nor between the vaccinated and control groups (Table 1).

IBDV antibodies in chickens vaccinated at day old with either vaccine were not significantly different except at 4th, 5th and 7th week's post-vaccination and all groups showed significant differences from the control group at 1, 4, 5, 6 and 7 weeks post-vaccination. At 10 days vaccination with IBDV vaccine, the two vaccinated groups showed significantly different antibody titers against IBDV except in the 5th week. Also, at 18 days vaccination there was no significant difference between both groups except at the 3rd week post-vaccination (Table 1).

These results are consistent with previous studies (Wood *et al*., 1983). At hatching, passive IBDV-specific Serum Neutralization (SN) antibody titers were found to be higher in the progeny of breeders given the IBDV-containing oil emulsion vaccine (Knoblich *et al*., 2000). Progeny mean IBDV specific SN antibody titers at 10, 25 and 40 days of age are shown in Table 1. At 10 days post-hatching, chicks from IBD-OEV breeders presented the highest ($p<0.05$) IBDV-specific antibody titers. At 25 days post-hatching, chicks from unvaccinated breeders and those vaccinated *in ovo* had higher antibody titers as compared to chicks from IBD-OEV vaccinated breeders ($p<0.05$).

These findings are in agreement with previous researches evaluating the interference of maternal antibodies on vaccinal IBDV infection (Knoblich *et al*., 2000; Alam *et al*., 2002; Rautenschlein *et al*., 2005). Indeed, Sharma (1985) found similar *in ovo* responses, with low passive antibody titered progenies at hatching presenting low response to day old subcutaneous vaccination and progenies with no IBDV-specific antibody titers presenting better protection to very virulent IBDV (vvIBDV) challenge. At 40 days of age, chicks from unvaccinated breeders responded with higher titers ($p<0.05$), irrespective of vaccination route (*in ovo*, subcutaneous or drinking water). The results from this study showed that progeny derived from breeders vaccinated with oil emulsion vaccine and vaccinated at hatching were consistent with those of Knoblich *et al*. (2000), Kumar *et al*. (2000) and Ahmed *et al*. (2003).

In ovo vaccination titers were similar to those described by others (Coletti *et al*., 2001). *In ovo* vaccinated IBD-OEV breeder progenies had lower responses ($p<0.05$) at 25 and 40 days of age as compared to unvaccinated breeders progenies. These results agree with previous findings (Coletti *et al*., 2001) and suggest that high titers of IBDV-specific passive antibodies negatively interfere with intermediately attenuated IBDV vaccine strain replication.

Bird's performance: In 21 days old chickens vaccinated at day 1, the weight gain in both vaccinated groups was not higher ($p<0.05$) than that of the control groups and similar results were recorded in chickens vaccinated at 10th day (Fig. 1). Chickens vaccinated at 18 days showed significantly increased body weight in group V1 (18) vaccines as compared to control group and V2 (18). A possible explanation is that detrimental effects of vaccinal infection may have been prevented by the higher titers of IBDV-specific passive antibodies in the chickens.

At the age of 42 days, chickens vaccinated with V1 at 1 and 10 days and with V2 at 1 day old showed no significant difference in body weight but both were better than control group one ($p<0.05$). However, vaccinated group at 18 days in V2 (10) showed significant increase in body weight in comparison to all other groups and the control. The average body weights at 42 days old chickens in group V2 (10) showed significant difference

Table 2: Weight of lymphoid organs at 21 and 42 days old post vaccination with two IBDV vaccines and RT-PCR in bursa

		Average weight ratios			
Groups	Sample (days)	Thymus	Bursa	Spleen	RT-PCR in bursa
V1(1)	21	0.20±0.020^{b}	0.061±0.006^{b}	0.18±0.011^{a}	-
	42	0.27±0.070^{e}	0.092±0.029^{ab}	0.08±0.019^{a}	-
V2(1)	21	0.30±0.042^{a}	0.067±0.013^{b}	0.20±0.040^{a}	-
	42	0.34±0.012^{cde}	0.064±0.006^{b}	0.21±0.014^{a}	-
V1(10)	21	0.25±0.040^{ab}	0.070±0.011^{b}	0.11±0.022^{bc}	-
	42	0.32±0.032^{de}	0.123±0.029^{a}	0.07±0.002^{a}	-
V2(10)	21	0.34±0.037^{a}	0.222±0.045^{a}	0.08±0.017^{cd}	-
	42	0.34±0.037^{bc}	0.221±0.045^{b}	0.09±0.017^{a}	-
V1(18)	21	0.30±0.026^{ab}	0.049±0.003^{b}	0.15±0.017^{ab}	-
	42	0.39±0.200^{bcd}	0.128±0.018^{a}	0.07±0.002^{a}	+
V2(18)	21	0.25±0.027^{ab}	0.189±0.022^{a}	0.06±0.003^{d}	-
	42	0.54±0.025^{a}	0.048±0.002^{b}	0.07±0.003^{a}	+
C	21	0.27±0.026^{ab}	0.177±0.014^{a}	0.07±0.008^{cd}	-
	42	0.45±0.059^{ab}	0.050±0.006^{b}	0.08±0.003^{a}	-

$^{a-d}$Values with different in the same row with different superscript differ significantly ($p<0.05$)

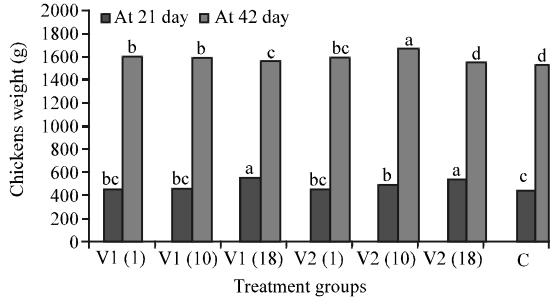

Fig. 1: Effect IBDV vaccine on average body weight of chickens at 21 and 42 days old. Each bar represent all birds in the same groups with different letter differ significantly ($p<0.05$)

compared to all groups (Fig. 1) while chickens vaccinated with D78 vaccine at 18 days had higher body weight than transmune vaccinated and control groups ($p<0.05$).

In the present study, 1 and 10 days old vaccinations caused reduction in body weight compared to 18 days old vaccination at 21 days old. However, body weight at 42 days old group V2 (10) showed significant differences compared to other groups while all groups showed significant differences from control one ($p<0.05$). Other researchers reported similar results in chickens vaccinated at 15 days old (Michell *et al*., 2009).

Lymphoid organs and RT/PCR: At 21 days old, the thymus showed no significant difference with regards to organ; body weight ratios between all groups. In 42 days olds, there was also no significant difference in organ; body weight ratios between all groups except that V2 (18) was higher than all other treatments except the control. However at 21 days, the bursa body weight ratio showed significant difference between V1 at (1, 10 and 18 days) and V2 (1) with V2 (10 and 18 days) vs. control group (Table 2). At 42 days old, bursa showed no significant difference between all groups post-vaccination. There was no significant difference between V1 (1, 10 and 18) and V2 (1) in spleen weight ratio to body weight while chickens in groups V2 (10, 18) showed significant differences in spleen ratio as compared with other groups at age 21 days. At age 42 days, no significant differences in spleen weight ratio were recorded between all groups. The bursa to body ratio in group V1 vaccinated with IBDV (D78) vaccine at 1, 10 and 18 days old and V2 vaccinates with (transmune) at 1 day old was significantly lower ($p<0.05$) than control and chicken vaccinated with V2 at 10 and 18 days old in 21 days post-vaccination. However at 42 days, control group and V2 vaccine showed significantly lower weight ratio than other groups.

The RT-PCR test for IBDV in lymphoid organs showed positive results for RNA detection with both vaccines at 42 days old in chickens vaccinated at 18 days olds post-hatching while no virus was detected in other organs which agrees with other researches (Corley *et al*., 2001). The present results are in disagreement with the finding of Ivan *et al*. (2005), especially with regards to detection of IBDV at 21 days post-vaccination which was negative in this study. In the present study, all samples of bursa, spleen and thymus were preserved in 10% formalin and this could have affected virus detection in these organs and probably, the virus could not multiply in the chickens until the end of the experiment.

CONCLUSION

It is derived from these results that both vaccines induce an adequate positive response in performance in chickens vaccinated subcutaneously at 1, 10 and 18 days old and that transmune vaccine caused less damage of the bursa compared to control group. Moreover, chickens vaccinated at 10 or 18 days showed better immune response to IBDV vaccination.

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