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RESEARCH ARTICLE

Efficacy of Live attenuated and Inactivated Oil Emulsion Infectious Bursal Disease Virus Vaccines in Broiler chicks

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ABSTRACT

This study was carried out with the aims to evaluate the efficacy of indigenous live and inactivated Infectious bursal disease virus (IBDV) vaccines in broilers. Two hundred and fifty (250), a-day-old broiler chicks divided into five groups (A-E) were immunized with live and inactivated vaccine at varying ages. Live vaccine was given to group A (at 8 days post hatch), B (at 8, 15 days post hatch), C (at 8, 15 and 23 days post hatch) and D (at 8 days post hatch). In addition group D received a booster dose of inactivated vaccine at 21 days of age, while group E served as control. Antibody titers were measured via Agar Gel Precipitation (AGP) test and ELISA, while the degree of protection against the virulent strains of IBDV was also recorded. Results showed that vaccine program adopted for group C and D produced significantly ($P<0.05$) higher antibody titer as compared to other groups. While a significant ($P<0.05$) difference in antibody titers was observed between group A and B while no considerable antibodies were detected in group E. The response to challenge dose was recorded as the difference of lesions in bursa, pectoral muscles or other visceral organs with the exception of group C and D. The study suggests that broiler chicks may be vaccinated at days 8, 15 and 23 with live attenuated vaccine or live attenuated vaccine followed by inactivated vaccine at days 8 and 21 that could provide an adequate protection against the virulent form of IBDV.

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INTRODUCTION

Infectious bursal disease (IBD), an immune-suppressive disease of chickens leads to heavy economical losses to poultry industry (Lukert and Saif, 2003; Mahmood *et al*., 2006; Uddin *et al*., 2010). IBD was successfully controlled through vaccination using classical strains, however, in 1988 the emergence of very virulent form in Europe and variant strains in United States caused a sub-clinical immune-suppression despite of vaccination (Rautenshlein *et al.,* 2005). The variant isolates differ pathologically and serologically from classical IBDV strains and they contain different neutralizing epitopes which causes vaccination failures. Therefore vaccines prepared from indigenous strains have been observed to provide better protection due to more antigenic relatedness (Hsieh *et al*., 2010; Rojs *et al.,* 2011). A part from vaccines, the vaccination programs also play an important role in providing adequate protection but may vary from country to country and area to area (Block *et al.,* 2007). In addition, the vaccination program is also influenced by pathogenicity of viral challenge, placement program, density and diversity of the poultry population in the area of operation, level of biosecurity and ability of a vaccine to produce stress (Tsukamoto *et al.*, 1995; Alam *et al.*, 2002; De Wit, 2003). Maternal antibodies (MA) have also been reported to interfere with the vaccination program against IBD (Al-Natour *et al.*, 2004). Despite of heavy vaccination clinical outbreaks are reported in Pakistan and only 10% of the farmers use laboratory services for monitoring the immune status in their flocks.

Present study was designed to determine the efficacy of indigenous live attenuated and inactivated oil emulsion IBDV vaccines and to recommend an effective vaccination program for broilers to suit poultry industry in Pakistan. Besides these, another criterion of the present study was to reveal the maternal immune status in local broiler chickens.

MATERIALS AND METHODS

IBDV Vaccine: Indigenous live-attenuated and inactivated vaccines prepared at Sindh Poultry Vaccine Centre (SPVC), Karachi, Pakistan from NL3/SPVC/2003 a virulent strain of IBDV (Lone *et al.*, 2009) were used in this study. Two hundred and fifty, a-day-old commercial broiler chicks purchased from a local hatchery were immunized using these vaccines (Table 1).

Challenge study and serology: Blood samples were collected randomly from 15 chicks in each group pre vaccination at days 2, 4, 6, and 8 days prior to vaccination and up to seven weeks of age post vaccinations. The vaccinated and control birds were challenged after 6 weeks with virulent field strain NL-3 /SPVC/2003 of IBDV via eye drop route. Birds were bled daily from each group, necropsied and gross pathological lesions recorded on bursa, pectoral muscles and spleen. The bursa to body-weight (BW) ratio and spleen to body weight ratio was calculated as described by Rauteschlin *et al.* (2003).

Serological testing of the collected samples were performed using AGPT and ELISA (Trop-Bio, Pty, Limited, James Crook University, Australia) and data analyzed using one way ANOVA.

RESULTS

The present study was conducted to determine the efficacy of indigenous live attenuated and inactivated IBDV vaccines and to recommend an effective vaccination program to protect broiler chickens against vvIBDV.

Vaccine efficacy study: The results showed that broiler chickens of group C and D showed significantly ($p<0.05$) higher antibody titers based on AGP and ELISA as compared to other groups, while a non significant difference was observed between the birds in group A and B (Table 2; Fig 1). Higher antibody titers were observed at week 5 and 6 in chickens of group C and D respectively while low levels were observed in group A and B. The pattern of antibody rise between group C and D was similar when compared at 5 and 6 weeks of age. It was also observed that the maternal antibodies were undetectable by AGP test and considerably low by ELISA test at 8 days post hatch of broiler chickens (Table 2). A higher body weight was observed in chicks of group A and D in comparison to group B and C while all treated groups (A, B, C, D) weighted less (100, 200, 300 and 100g, respectively) when compared with the control group (data not shown).

Challenge study: The results show that the chickens of group A when challenged at week 7 of age, showed marked lesions in thigh, pectoral and breast muscles, 4th to 7th day of post-challenge (Table 3). However, no marked splenomegaly and bursal atrophy was observed. While group (B, C, D) showed no lesions in pectoral and thigh and breast muscles and no abnormality was observed in bursa and spleen (Table 3). However, 90% of the chickens in control group (E) showed marked hemorrhagic lesions pectoral, breast and thigh muscles with atrophied bursa and enlarged spleen (Table 3). Further all challenged birds have significantly lower bursa/ body weight ratio than non-vaccinated (Table 4). A reduced bursal size was observed in groups who had received booster or tertiary dose of live attenuated vaccine as compared to chickens that received single dose of live attenuated vaccine or live vaccine followed by inactivated vaccines (Table 4).

DISCUSSION

IBDV is one of the most common diseases of commercial poultry in Asia. Economically poultry industry faces great losses due to the introduction of new antigenic or pathogenic strains of IBDV. Vaccination is

Table 1: Plan of work

Type of Vaccine	Age (Days)	Groups					Route of Administration
		A	B	C	D	E	
Live Attenuated Vaccine	8	√	√	√	√	-	Eye drop
	15	-	√	√	-	-	
	23	-	-	√	-	-	
Killed Vaccine	21	-	-	-	√	-	Sub-cutaneous

√ = Indicates the day of vaccination; Each group contains 50 chicks

Table 2: Agar Gel Precipitation (AGP) results up to six weeks post vaccination

Groups	Age in Days post vaccination					
	7	14	21	28	35	42
A	-	+	+	+	+	+
B	-	+	+	++	+	++
C	-	+	+	+++	++	+++
D	-	+	+	++	+++	+++
E	-	-	-	-	-	-

- = No precipitation lines; + = Precipitation lines; ++ = Specific; precipitation lines; +++ = Highly specific precipitation lines.

Table 3: Gross Pathological Lesions Recorded in Broiler Chickens Post Challenge Virulent Strain, NL-3/SPVC/2003 of Infectious Bursal Disease Virus

Group	Lesions recorded	Post-mortem findings after challenge (Days)						
		1	2	3	4	5	6	7
A	P M	-	-	-	+	+	++	+
	T M	-	-	-	+	+	++	++
B	P M	-	-	-	-	+	-	-
	T M	-	-	-	-	-	-	-
C	P M	-	-	-	-	-	-	-
	T M	-	-	-	-	-	-	-
D	P M	-	-	-	-	-	-	-
	T M	-	-	-	-	-	-	-
E (Control)	P M	++	++	++	+++	+++	+++	+++
	T M	+	++	+++	+++	+++	+++	+++

Each group contains 30 chicks; - = No Lesions; + = Lesions; ++ = Prominent lesions; +++ = Highly prominent lesions; PM = Pectoral Muscles; TM = Thigh Muscles.

Table 4: Lymphatic organs (bursa and spleen) vs body weight ratios in vaccinated and post challenged birds (n=50)

Ratios	Treat-ments	Groups			
		A	B	C	D
Bursa vs BW (Post Vaccination)	1	1.52±0.18^{a}	1.38±0.11^{a}	1.29±0.40^{a}	1.52±0.08^{a}
	2	1.75±0.15^{b}	1.76±0.17^{b}	1.74±0.23^{b}	1.71±0.19^{b}
Bursa vs BW (Post Challenge)	1	1.39±0.22^{a}	1.40±0.21^{a}	1.36±0.22^{a}	1.60±0.21^{a}
	2	0.94±0.56^{b}	1.14±0.20^{b}	0.80±0.27^{b}	1.00±0.22^{b}
Spleen vs BW (Post Challenge)	1	1.23±0.25^{a}	1.15±0.33^{a}	1.20±0.27^{a}	1.23±0.18^{a}
	2	2.25±0.80^{b}	1.75±0.50^{b}	1.90±0.45^{b}	2.20±0.50^{b}

Mean±SE; 1=Vaccinated; 2=Unvaccinated; Different superscript letters indicate a significant ($P<0.05$) difference within the group; BW = Body weight.

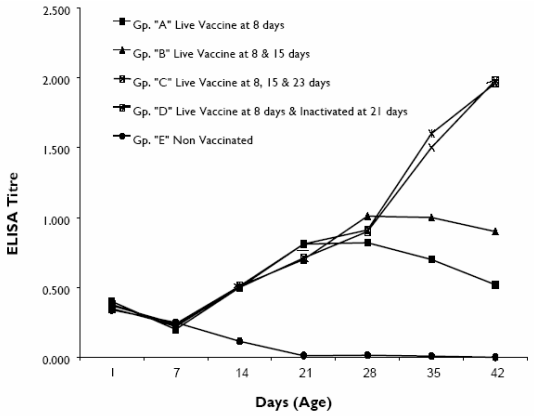

Fig. 1: ELISA antibody titre of broiler chickens vaccinated with different regimes of indigenous IBDV live and inactivated vaccine.

the only preventive measure against the disease. A part from live attenuated vaccine the killed vaccine is more commonly being employed in commercial broiler farming. This study has revealed that primary immunization of flock with live vaccine followed by booster through inactivated vaccine increase the chances of protection against IBDV.

Passive immunity against IBDV has been reported to interfere with the vaccination program of IBDV (De Wit, 2003; Rautenschlein *et al.,* 2005). Day old chicks have high levels of maternal antibodies (Alam *et al*., 2002) that protect them up to 3 weeks of age, but reduce their immune response to active immunization thus an optimum vaccination time for each flock must be determined for effective control of vvIBDV (Kenji *et al*., 1995). In contrary the maternal antibodies can be detected via AGP and ELISA up to 4 and 8 days respectively during this study which is in agreement to the studies by Yong *et al.* (1995). Often, in Pakistan Immune status prior to vaccination is not determined by poultry farmers. In routine they immunize at an age of 13 days through live attenuated vaccine followed by a booster dose of inactivated vaccine at 35 days of age. This practice is less effective in controlling the infection since maternal antibody level plays an important role in primary immunization as described by Van den Berg and Meulemans (1991). It has been reported that, contrary to classical IBDV; maternal antibodies could not provide protection to broilers and layers if exposed to vvIBDV challenge (Mardassi *et al.,* 2004). Similar results have been observed during this study when birds of group A and B showed severe hemorrhagic lesions in pectoral muscles. Moreover, splenomegaly and extensive hemorrhages in 90% of control birds were observed in pectoral muscles, breast muscles along with hemorrhages and gelatinous exudate in bursa. Whereas, all vaccinated birds have significantly lower BF/ body weight ratio than non-vaccinated birds. Therefore the findings are in agreement that the protection level against IBDV challenge varies on the basis of different vaccination programs (Van den Berg and Meulemans, 1991).

The emergence of various new strains of IBDV has complicated the protection against the IBD infection (Knoblich *et al.,* 2000). The ability of vaccine virus to protect against variant challenge is associated with both, the dose and strain of challenge and vaccine viruses. Selection of vaccines from the 'mild', 'intermediate' and low attenuation or 'hot' classification depends on the management and stock-related factors, level and uniformity of maternal antibody transfer, virulence of field virus strains, and risk of challenge (Lukert and Saif, 2003). Successful control of vvIBDV is achieved by administering less attenuated ('hot') vaccine strains capable of stimulating immunity in the presence of maternal antibody. Since the vaccines were used in this study were prepared from indigenous strain of IBDV, therefore on challenge it provided adequate protection. Similar has been reported earlier when broiler chicks were administered vaccines in the presence of maternal antibody were protected against vvIBD challenge when administered at 7-10 days of age (Van Den Berg and Meulemans, 1991; Zaheer and Akhtar, 2003; Xuemei *et al.,* 2010).

Decrease weight gain was also noted in broiler chicks who received single or two booster doses of live IBD vaccines as compared to group D received a booster dose of inactivated vaccine. This might be due to the stress caused by live virus vaccines in commercial chickens. Banda *et al.* (2008) also reported that route and doses of IBD vaccines affect the weight gain in broiler Chickens.

The archetype of present investigation is that, low levels of maternal antibodies were found in commercial broiler chickens at 8 days of age which is in contrary to previous studies. Repeated vaccination with live vaccine may cause a significant decrease in weight gain. Therefore, administration of live vaccine at 8 days followed by a booster dose of inactivated oil emulsion vaccine at 21 days (group D) is recommended for commercial broilers since it can provide adequate protection against the virulent form of IBDV with minimum adverse effects.

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