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Evaluation of *In Ovo* Vaccination Against Infectious Bursal Disease Virus in Commercial Broilers in Pakistan

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ABSTRACT

The present study was conducted to evaluate the *in ovo* route of vaccination against infectious bursal disease virus(IBDV).A total of 160, 18 days old embryonated chicken eggs were divided into four groups i.e. A,B,C, and D having 40 eggs in each group. Three different IBD virus vaccines i.e. intermediate strain (D-78), hot strain (Bursin plus) and a locally prepared egg adapted vaccine (24th passage) were inoculated through amniotic cavity route in the eggs of group A, B and C respectively. The group D was kept as unvaccinated control. Eggs were allowed to hatch and experimental chicks of each group were reared separately. The hatchability was 93, 83.33,90 and 97% in group A, B, C and D, respectively. The geometric mean titer of antibodies (GMT) was highest (146.96) in group B followed by group A and C (127.94) at 14 days post hatching. The GMT was 55.69, 63.97 and 55.69 in group A, B and C at 21 days post hatching. The GMT of IBDV was highest in group B i.e.64.00 in bursa and 32.00 in spleen at 0 day post-hatching followed by group A and C, respectively. The virus titer then decreased gradually up to 15 days post-hatching. The titers were higher in bursa then spleen. The protection against challenge with virulent IBDV was 100, 100, 90 and 17% in group A, B, C and D, respectively. The overall result proved that *in ovo* vaccination was highly safe and effective in protecting the young chicks against IBD in their early life.

Key Words: *In ovo* vaccination; Infectious bursal disease; Amniotic cavity route

INTRODUCTION

Infectious bursal disease (IBD) also known as Gumboro disease is an acute, highly contagious, viral infection of young chickens that has lymphoid tissue as its primary target with a special predilection for bursa of Fabricious. The disease is caused by infectious bursal disease virus which is a member of genus *Avibirna virus* of family *birnaviridae*. The viral genome consists of two segments of dsRNA, designated as A and B, which are enclosed within an icosahedral, non-enveloped capsid of 60nm in diameter (Lukert & Saif, 2003). Gumboro disease virus exist in two antigenically distinct serotypes i.e. I and II. The only serotype I which displays a wide variation of pathogenic potential is virulent for chickens whereas serotype II is present in turkeys. Vaccination is the principle method of controlling IBD. Field exposure to the virus or vaccination with either live or killed vaccines is various means of antigenic stimulus leading to the induction of active immunity (Lukert & Saif, 1991). In poultry industry, hand inoculation of broiler chicks on the day of hatching is rapidly giving way to the automated vaccination of embryos by injection through egg shell on the 18th day of incubation, when eggs are routinely transferred to hatching trays. The wide spread application of this vaccinal technique may protect chicks in the first week of age against the infectious bursal disease (Coletti *et al*., 2001). The concept of embryo vaccination is based upon the fact that chicks develop immunological maturity well before hatching. They are fully immuno competent at 18th day of embryonation.

In ovo vaccination is used successfully against Marek's disease and studies have been performed to evaluate the efficacy of this kind of vaccination against Newcastle disease (ND), infectious bronchitis (IB) and infectious bursal disease (IBD). Particularly for IBDV, it is difficult to choose a rational programme, the active and passive immunity must both be considered in the development of immunization programme. Passive immunity is of critical importance because chicks have to be protected throughout the early period of life, when they are more susceptible to immunosuppressive effect of IBDV (Coletti *et al*., 2001). However, when progeny is vaccinated at an early age with a mild or highly attenuated live vaccine, high level of maternal antibody may interfere with the development of active immunity. Because of maternal antibody interference associated with lack of uniform antibody titer in progeny, repeated vaccinations are needed until maternal antibody wanes. Therefore it would be advantageous to stimulate an early immune response in chickens with an *in ovo* vaccine.

Present study was conducted to evaluate the efficiency of *in ovo* route of vaccination against infectious bursal disease using different IBDV vaccines.

MATERIALS AND METHODS

Source of embryonated chicken eggs and IBD virus. One hundred and sixty, 18 days old, live embryonated chicken eggs of commercial broiler breeder were procured from poultry research centre, University of Agriculture, Faisalabad. Two imported commercial IBDV vaccines i.e. intermediate strain (D-78) (Neuva, Italy) and hot strain (Bursin plus) (Fort Dodge, USA) were purchased from local market. A locally prepared egg adapted IBDV vaccine (24th passage) was obtained from Department of Veterinary

Microbiology, University of Agriculture, Faisalabad.

Inoculation of chicken embryos. The eggs were candled to mark the air sac and head of the embryos and then air sac area was swabbed with 70% alcohol. The eggs were divided into four groups i.e. A, B C and D having 40 eggs in each group. Imported vaccines were reconstituted in normal saline. A 0.1 mL of each of the three vaccines i.e. D-78, bursin plus and egg adapted was inoculated into the embryonated eggs of group A, B and C, respectively through amniotic cavity route (Jochemsen & Jeurissen, 2002). The eggs of group D were kept as uninoculated control. The inoculated eggs were sealed with wax. All the eggs were kept in incubator till hatching.

Experimental chicks. Hatchability percentage in each group was recorded. The hatched chicks were identified by color marking. All the groups of experimental chicks were separately brooded and kept separately in experimental animal house of the Department of Veterinary Microbiology. However, all the chicks were offered with same feed and water.

Measurement of humoral immune response. Blood samples were collected from 5 chicks at random in each group at 0, 7, 14 and 21 days post-hatching. The serum was separated and heat inactivated in water bath at 56°C for 30 minutes. The antibody levels against infectious bursal disease virus (IBDV) were determined in each group using indirect haemagglutination (IHA) test (Hussain *et al*., 2003).

Antigen titeration in bursa and spleen. Five chicks were slaughtered from each group at 0, 5, 10 and 15 days post-hatching. Spleen and bursae were collected and processed for virus isolation. A 10% W/V suspension in normal saline containing penicillin (100IU mL^{-1}) and streptomycin (1000µg mL^{-1}) was prepared separately for each sample. The homogenates were centrifuged at 1500 rpm for 10 minutes. The supernatants were collected and treated with chloroform (1:1) to remove the tissue debris (Reddy *et al*., 1997). The purified supernatants were subjected to reverse passive haemagglutination (RPHA) test for titeration of IBD virus using known hyperimmune serum (Rajeswar & Dorairajan, 1999).

Challenge protection test. A 50% of remaining birds in each group were challenged with 0.1 mL of locally isolated and characterized virulent IBD virus at 21st day post-hatching. The clinical signs and lesions in bursa and other organs were carefully examined uptill 7 days post-challenge. The morbidity and mortality was recorded and protection percentage was calculated in each group.

RESULTS AND DISCUSSION

Present study was successfully carried out for the first time in Pakistan to protect the young chicks against IBD during early days of life by strengthening the maternal antibody levels through *in ovo* vaccination. The vaccination was done at 18th day of incubation through amniotic cavity route. The hatchability was 93, 83.33. 90 and 97% in group A, B C and D respectively. There was no significant difference in hatchability percentage between vaccinated and non-vaccinated groups. Similar results were obtained by Worthington *et al*. (2003).

The antibody titers were measured by indirect haemagglutination (IHA) test. The titer ranged from 16 to 32 in group A and B and from 8 to 32 in group C at zero days. At day 7 and 21 post-hatching, titers were highest in all three groups ranging from 64 to 256. The geometric mean titer (GMT) was also highest in group B i.e. 24.24, 73.49, 146.96 and 63.97 at 0, 7, 14 and 21 days post-hatching, respectively. It was followed by group B, C and then D. The cumulative mean titer (CMT) was 55.69, 63.67, 51.67 and 9.52 in group A, B, C and D, respectively. The birds of group D which were kept as unvaccinated control did show some maternal antibody titers which decreased gradually (Table I). The results of IHA revealed that all three vaccines administered through *in ovo* route showed high antibody titers. Normally, the maternal antibodies are completely catabolized in case of IBD and titer may go to zero by the end of third week, which is the most susceptible age for IBD in broilers. With *in ovo* vaccination, the GMT and CMT were still very high and protective at 21 day post-hatching (Lukert & Saif, 1991). The bursin plus vaccine showed highest antibody titer, because it contains a hot strain of virus which can produce high antibody titer even in the presence of maternal antibodies but hatchability percentage was comparatively low in this group.

The IBDV was also titerated from bursa and spleen at 0, 5, 10 and 15 days post-hatching using RPHA test. The virus titer was found more in bursa than spleen. The maximum IBDV was detected in bursa and spleen at 0 day post-hatching (3rd day post *in ovo* vaccination). The virus titer then decreased gradually upto a very low titer at day 15 post-hatching. The unvaccinated birds of group D did not show any significant virus titer. The GMT in bursa and spleen of the birds of different groups are shown in Table II.

Ahmad and Sharma (1993) also detected IBDV in spleen, intestine, liver and bursa between 4 to 10 days post inoculation (PI). The peak titer was present between 4 to 6 days PI. Replicating IBDV was also found in several organs after 24 h as when it was injected at 18 days of embryonation (Jochemsen & Jeurissen, 2002).

Similar observations were also recorded by Michelle *et al*., 2001, who detected IBDV in lymphoid tissues after *in ovo* vaccination of SPF embryos. High titer was found on day 3 post *in ovo* vaccination (PIOV) and dropped considerably by 9 day of PIOV. The decrease in viral antigen may correlate with the development of immune response. Tsukamoto *et al*. (1995) also observed that maximum antigen titer was found in bursa which maintained for 1-3 days postinoculation.

The 50% of the remaining birds were challenged at 21st day with virulent IBDV having EID_{50} $10^{-5.54}$. The results showed that no bird died or became sick in group A and B upto 7 days post-challenge. So protection was 100% in group A and B followed by 90% in group C. In group D

Table I. IHA antibody titer in different groups at 0, 7, 14 and 21 days post-hatching

Groups/Sample		Days post-hatching				CMT
	No.	0	7	14	21	
	1	16	64	64	64	
	2	16	64	128	32	
A	3	32	32	256	64	
	4	16	64	64	128	
	5	32	128	256	32	
	GMT	21.1	63.97	127.94	55.69	55.69
	1	16	128	256	128	
	2	32	64	64	32	
	3	32	128	128	64	
B	4	16	32	128	64	
	5	32	64	256	64	
	GMT	24.24	73.49	146.96	63.97	63.97
	1	8	32	64	32	
	2	16	64	64	128	
C	3	16	128	256	64	
	4	32	64	128	64	
	5	32	32	256	32	
	GMT	18.37	55.69	127.94	55.69	51.97
	1	16	8	4	2	
	2	32	16	16	8	
	3	8	8	16	8	
D	4	8	8	8	8	
	5	16	32	8	4	
	GMT	13.93	12.12	9.19	5.28	9.52

A= D-78, B=Bursin plus, C= Egg adapted, D= Control; GMT=Geometric Mean Titer, CMT=Cumulative Mean Titer

Table II. The GMT of IBD virus in bursa and spleen at 0, 5, 10 and 15 days post-hatching

Days	Organs	Groups/GMT			
		A	B	C	D
	Bursa	63.52	64.00	58.52	0.00
0	Spleen	31.80	32.00	31.00	0.00
	Bursa	42.24	45.69	39.37	1.32
5	Spleen	22.52	27.56	22.52	1.21
	Bursa	23.57	24.67	18.79	1.05
10	Spleen	8.00	8.57	7.47	1.00
	Bursa	11.27	13.27	10.69	0.00
15	Spleen	3.82	4.00	2.89	0.00

which was unvaccinated control, birds showed severe signs and lesions of IBD including sever haemorrhages on bursa and thigh muscles and protection was only 17%. The results are in line with those of Sharma *et al*. (1995) who reported that *in ovo* vaccination provided 93.25% protection against challenge with very virulent strain of Marek's disease virus.

On the basis of the results of present study it was concluded that *in ovo* route of vaccination against IBDV is quite safe and efficacious in protecting the young chicks during the early stages of their life. It not only provoked the immune response of young chicks in terms of higher antibody titres but also provided 100% protection against challenge.

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