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IMMUNOGENIC RESPONSE WITH EFFICACY OF CERTAIN GUMBORO VACCINES IN BROILER CHICKENS

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ABSTRACT

The research work was carried out to determine the immunogenic response with efficacy of six commercial Gumboro vaccines (Nobilis® Gumboro D78 and Nobilis® Gumboro 228E, Intervet, The Netherlands; Bur-706®, Merial, France; TAD Gumboro vac® and TAD Gumboro vac forte®, Lohmann Animal Health, Germany; Bursaplex™, Merial Select, Inc. Gainesville, USA) under experimental condition in broiler chickens during the period from November 2002 to May 2003. The chickens of two groups were vaccinated with Nobilis® Gumboro D78 and Nobilis® Gumboro 228E at 14 days of age with a booster dose at 21 days of age and were challenged at 40 days of age with vvIBDV. TAD Gumboro vac® and TAD Gumboro vac forte® were inoculated into the two groups of chickens at 14 and 28 days of age and the chickens were challenged at 35 days of age with vvIBDV. Bur-706® vaccine was given to the chickens of another group at one-day-old and 14 days of age and the chickens were challenged at 28 days of age. One group of chickens was vaccinated with Bursaplex™ vaccine at 1-day-old with no booster dose and was challenged at 21 days of age. The percentage of protection in birds receiving Nobilis® Gumboro D78 and Nobilis® Gumboro 228E vaccines by intraocular route was 92.30 and 96.29 respectively, whereas the percentages of protection in birds receiving TAD Gumboro vac®, TAD Gumboro vac forte® and Bur-706® vaccines were 95.65, 100 and 95.83 respectively. Bursaplex™ provided 100% protection against challenge with vvIBDV. Chickens vaccinated with Nobilis® Gumboro D78, Nobilis® Gumboro 228E, TAD Gumboro vac®, and Bur-706®, showed significant ($p < 0.05$, $p < 0.01$) decrease in ELISA antibody titre up to the day of challenge infection. Bursaplex™ vaccinated group also showed significant decrease in ELISA antibody titre by 7 days ($p < 0.05$) and by 14 and 21 days ($p < 0.01$) after primary vaccination. In case of TAD Gumboro vac forte®, 7 days (839 ± 219.34) and 14 days (258 ± 44.80) after primary vaccination, the ELISA antibody titre significantly ($p < 0.01$) decreased but 7 days after booster vaccination, the ELISA antibody titre significantly ($p < 0.01$) increased (1299 ± 37.51). All the control sera revealed significant ($p < 0.01$) decrease of ELISA antibody titre up to day of challenge infection. There was an insignificant increase in ELISA antibody titre 7 days after booster vaccination in case of unvaccinated control groups for TAD Gumboro vac® and TAD Gumboro vac forte® only. Nobilis® Gumboro D78, TAD Gumboro vac® and Bur-706® vaccinated groups revealed significant ($p < 0.01$) increase of ELISA antibody titre 7 days post-challenge while Nobilis® Gumboro 228E and Bursaplex™ vaccinated groups showed significant ($p < 0.05$) increase of antibody titre after 7 days of challenge. Insignificant increase of antibody titre was recorded in TAD Gumboro vac forte® vaccinated group. It may be concluded that TAD Gumboro vac forte® (Intermediate plus) and Bursaplex™ (Merial Select, Inc. Gainesville, USA) can be used to immunize the broiler birds sufficiently against IBD. However, it is necessary to estimate the optimum vaccination timing, i.e., to determine when maternal antibodies in chicks will decline to levels that the vaccines can overcome.

Key words: Gumboro vaccines, immune response, efficacy, broiler chickens

INTRODUCTION

The infectious bursal disease is endemic in the environment of commercial poultry operations and is considered to be among the most economically important infectious diseases affecting the poultry industry (Shane *et al.*, 1994). The morbidity of chicken infected with a classical IBDV strain was found higher than 80% while mortality was as low as 9 to 12% (Mohanty *et al.*, 1971) or may reach upto 50% (Brown and Grieve, 1992) in layer and 25% in broiler (Lukert and Hitchner, 1984). IBDV strains isolated from affected chickens induce severe clinical signs and high mortality (30% - 70%) in SPF chickens (Nunoya *et al.*, 1992; Tsukamoto *et al.*, 1992). Because of the environmental stability of the virus, inactivation is difficult, and, therefore, vaccination is considered the most practical means to control the disease (Knoblich *et al.*, 2000; Kouwenhoven and Bos, 1994), although the clinical outbreaks are also reported in vaccinated flocks (Muhammad *et al.*, 1996).

Vaccination is effective in controlling infectious bursal disease (IBD) when appropriate vaccination practices are followed. Infectious bursal disease (IBD) can be controlled by both live and inactivated vaccines. There are two kinds of live vaccines: those that have intermediate virulence and attenuated mild strains (Lukert and Saif, 1991). Although, high levels of neutralizing maternal antibody can interfere with the development of active immunity of the chickens to

vaccine virus (Abd-El-Aziz, 2000; Kibenge *et al.*, 1988), the intermediate vaccines are superior to the mild vaccines in giving immunity to commercial chickens with maternal antibodies, because intermediate vaccines are less affected by maternal antibodies (Tsukamoto, 1992). However, intermediate vaccines vary in virulence; some of them can induce severe bursal atrophy and immunosuppression in young chickens (Mazariegos *et al.*, 1990). In order to control IBD with live vaccines, it is critical to vaccinate commercial chickens that have maternal antibodies at the optimum time (Tsukamoto *et al.*, 1995). To determine the optimum vaccination time, researchers must first know the ability of each live vaccine to overcome maternal IBDV antibodies. Next, researchers must determine when maternal antibodies in chicks will decline to levels that the vaccines can overcome. Without this information about live vaccines, it is very difficult to control highly virulent IBDV. Recently, different companies have marketed a large number of imported IBD vaccines without knowing their efficacy, ability of them to overcome the maternal antibodies and adverse effects under local conditions in Bangladesh. Keeping the above perspectives in view, the present research work is being undertaken to determine the immunogenic response with efficacy of certain commercial Gumboro vaccines under experimental condition in broiler chickens.

MATERIALS AND METHODS

The research work was carried out to determine the immunogenic response with efficacy of six commercial Gumboro vaccines (Nobilis® Gumboro D78 and Nobilis® Gumboro 228E, Intervet, The Netherlands; Bur-706®, Merial, France; TAD Gumboro vac® and TAD Gumboro vac forte®, Lohmann Animal Health, Germany; Bursaplex™, Merial Select, Inc. Gainesville, USA) under experimental condition in broiler chickens during the period from November 2002 to May 2003.

Vaccination with Nobilis® Gumboro D78 and 228E

Of the 120 1-day-old commercial 'Vencobb' broiler chickens purchased from the Goalundo Hatcheries (Faridpur, Bangladesh), 77 chickens were divided into three groups (Group A: 27 chicks, Group B: 27 chicks, and Group C: 23 chicks) at the age of 14 days old. Each group was housed in a separate room with rice husks as litter. Feed (Quality Feeds Ltd., Dhaka) and fresh water were available *ad libitum*. Chickens of group C received no vaccine and served as controls. Chickens in groups A and B were vaccinated intraocularly with Nobilis® Gumboro D78 and Nobilis® Gumboro 228E (Intervet, The Netherlands) with a single dose (one drop in one eye) each at the age of 14 days and were boosted at 21 days of age according to the manufacturer's directions respectively. On day 40 of age, chickens from each group were challenged with the virulent IBD virus. After challenge, all the chickens were examined for signs of disease; examinations were performed either at the time of death or at 10 days post-challenge. Blood samples were collected weekly from each group and 12 days after booster vaccination and 7 days after challenge. Sera of each group were collected and stored at –20°C until tested.

Vaccination with Bur-706®, TAD Gumboro vac® and TAD Gumboro vac forte®

The experimental design was similar to that of previous one. Of the 120 1-day-old 'Arber Acres' broiler chickens purchased from the Goalundo Hatcheries (Faridpur, Bangladesh), 90 chickens were divided into five groups (Group A: 25 chicks, Group B: 25 chicks, Group C: 25 chicks, Group D: 5 chicks and Group E: 10 chicks). Each chick of Group A was immunized at the age of 1st (primary) and 14 (booster) days with Bur-706® (Merial, France); chicks of groups B and C were immunized at the age of 14 (primary) and 28 (booster) days with TAD Gumboro vac® and TAD Gumboro vac forte® (Lohmann Animal Health, Germany) respectively according to the manufacturer's directions while chicks of groups D and E were served as unimmunized controls. Chicks of group A (vaccinated with Bur-706®) and group D (unvaccinated control group for Bur-706®) were challenged at 28 days of age while vaccinated groups B, C and unvaccinated control group E were challenged at 35 days of age with virulent IBD virus. Chicks were observed for 7 days after challenge. To determine the antibody titre level, sera of each group were collected and stored at regular intervals at –20 °C until tested.

Vaccination with Bursaplex™

Briefly, a total of 30, 1-day-old 'Shaver Starbro' broiler chickens were purchased from Paragon Poultry Ltd., Dhaka, Bangladesh. These chickens were started on litter of rice husk in a house with concrete floors providing fresh drinking water and commercial pelleted feed (Quality Feeds Ltd., Dhaka) *ad libitum*. Bursaplex™ (Merial Select, Inc. Gainesville, USA), a live strain of bursal disease virus of chicken embryo origin in conjunction with bursal disease antiserum was inoculated subcutaneously into 25 chicks @ 0.2 mL per chicken at 1 day old of age. The remaining five chickens served as unvaccinated controls. At 21 days of age, both the vaccinated and unvaccinated groups of chickens were challenged with virulent IBD virus. The challenged birds were observed for two weeks. Sera of each group were collected and stored at regular intervals at –20 °C until tested.

Challenge infection

The virus suspension of a wild-type field strain (BD-3 Wt) of a Bangladeshi isolate of vvIBDV, BD-3/99 (Islam *et al.*, 2001) was used as challenge. For challenge infection, BD-3 Wt was passaged in chicks and bursa were collected to make 20% bursal homogenate diluting with PBS. Each chick received 100 µl of 20% bursal homogenate.

ELISA

The indirect enzyme-linked immunosorbnent assay was performed according to the manufacturer's (IDEXX Laboratories, Inc. Westbrook, Maine 04092, USA) instruction using pre-coated plates and pre-diluted, ready to use reagents and buffer. Incase of IDEXX ELISA, the titre was predicted from the absorbance value of 1:500 dilution of a serum using the formula supplied with the kit. Absorbance values were measured at 650 nm by computerized MDC, SPECTRA max 340 PC microplate reader and SOFTmax pro Microplate Analysis software (Labquip Exports (S) Ptc Ltd., Singapore).

Statistical analysis

The differences in the increase or decrease of the ELISA antibody titre and total leukocyte count of chicks of different groups at different ages were analyzed statistically with the help of Student's '*t*' test (Gupta, 1982) for significance.

RESULTS AND DISCUSSION

The comparative efficacy of certain Gumboro vaccines as determined by the state of protection in birds after challenge exposure is summarized in Table 1.

Table 1. Vaccination schedules and state of protection in broiler birds against infectious bursal disease immunized with certain commercial Gumboro vaccines

Parameters	Nobilis® Gumboro (Intervet, Netherlands)			TAD Gumboro (Lohmann Animal Health, Germany)			Bur-706® (Merial, France)		Bursaplex™ (Merial Select, Inc., USA)	
	D-78 (n=27)	228E (n=27)	Control (n=23)	vac (n=25)	vac forte (n=25)	Control (n=10)	Vaccinated (n = 25)	Control (n = 5)	Vaccinated (n = 25)	Control (n = 5)
Route of vaccination	IO	IO	–	IO	DW	–	IO	–	SC	–
Age of birds at vaccination (days)	14[a] 21[b]	14[a] 21[b]	– –	14[a] 28[b]	14[a] 28[b]	– –	01[a] 14[b]	– –	01 –	– –
No. of birds at vaccination	27	27	–	25	25	–	25	–	25	
No. of birds died after vaccination*	01	00	–	02	01	–	01	–	00	–
No. of birds at challenge	26	27	23	23	24	10	24	05	25	05
Age of birds at challenges (days)	40	40	40	35	35	35	28	28	21	21
No. of birds showing symptoms after challenge**	02	01	10	03	00	06****	02	05	00	05
No. of birds died after challenges***	02	01	06	01	00	00	01	01	00	01
No. of birds protected	24	26	17	22	24	10	23	04	25	04
% protected	92.30	96.29	73.91	95.65	100	100	95.83	80	100	80

[a]Primary vaccination, [b]Booster vaccination, *Two days after primary vaccination from D78® vaccinated group, three days after booster vaccination from TAD Gumboro vac® and TAD Gumboro vac forte® vaccinated groups and five days after primary vaccination from Bur-706® vaccinated group with unknown etiology. **Between 2 to 3 days after challenge, ***Between 4 to 5 days after challenge, ****60% birds from control group for TAD Gumboro vac® and TAD Gumboro vac forte® showed clinical signs with no mortality after challenge at 35 days of age but at postmortem examination at 42 days of age lesions were observed, IO = Intraocular, DW = Drinking water, SC = Subcutaneously.

It was observed that the percentage of protection in birds receiving Nobilis® Gumboro D78 and Nobilis® Gumboro 228E vaccines by intraocular route was 92.30 and 96.29 respectively, whereas the percentages of protection in birds receiving TAD Gumboro vac®, TAD Gumboro vac forte® and Bur-706® vaccines were 95.65, 100 and 95.83 respectively. Bursaplex™ provided 100% protection against challenge with vvIBDV. Birds of each vaccinated group, which succumbed to challenge exposure, showed swollen, edematous and slight haemorrhagic bursa. Only one bird from each vaccinated group of Nobilis® Gumboro 228E, TAD Gumboro vac®, Bur-706® and two from Nobilis® Gumboro D78 died after challenge infection whereas none from TAD Gumboro vac forte® and Bursaplex™ vaccinated groups. Some birds from all the vaccinated groups died before challenge for nonspecific cause except Nobilis® Gumboro 228E and Bursaplex™ vaccinated groups (Table 1). These observations support the earlier reports of Rosenbusch *et al.* (1990), Perera *et al.* (1996), Khaliel and Manakhly (1998) and Zhou and Li (2000) but contradict with the reports of Giambrone and Closser (1990) and Zorman and Cajavec (1997).

Among the vaccinated groups of chicken, the rate of protection was found higher (100%) in chickens those were vaccinated with TAD Gumboro vac forte® and Bursaplex™ followed by Nobilis® Gumboro 228E (96.29%). The protection rates of chicken vaccinated with Bur®-706 (95.83%) and TAD Gumboro vac® (95.65%) were also satisfactory, whereas the protection rate of chicken vaccinated with Nobilis® Gumboro D78 (92.30%) vaccine was not satisfactory compared to the other vaccines.

Chickens vaccinated with Nobilis® Gumboro D78, Nobilis® Gumboro 228E, TAD Gumboro vac®, and Bur-706®, showed significant ($p < 0.05$, $p < 0.01$) decrease in ELISA antibody titre up to the day of challenge infection (Table 2-4). Bursaplex™ vaccinated group also showed significant decrease in ELISA antibody titre by 7 days ($p < 0.05$) and by 14 and 21 days ($p < 0.01$) after primary vaccination (Table 5). This low ELISA titre even after booster vaccination may have occurred for two reasons: maternal antibodies might have interfered with the vaccine 'take', or the immunity induced by these live vaccines might simply be insufficient. These findings support the reports of Goddard *et al.* (1994), Knoblich *et al.* (2000) and Alam *et al.* (2002).

Table 2. Antibody titre detected by indirect ELISA in broiler chickens immunized against IBD with Nobilis® Gumboro vaccines (Intervet, Netherlands)

Vaccine	Pre-vaccination (n = 4)[#] 14 days[1]	Post-vaccination (n = 4)			Post-challenge (n = 4) 47 days
		Post-primary	Post-booster		
		21 days[2]	28 days	40 days[3]	
Nobilis® Gumboro D78	759-2818 1883 ± 786.22	437-3631 1681 ± 1151.89	0-550 272 ± 194.48**	15-229[a] 136.67 ± 89.79**	355-2089 1539.80± 629.46**
Nobilis® Gumboro 228E	759-2818 1883 ± 786.22	245-2818 1668.25 ± 925.61	69-1258 661 ± 489.70**	32-2138 717.75 ± 845.27	851-2754 1512.8 ± 660.17*
Control	759-2818 1883 ± 786.22	145-1585 948.5 ± 647.36*	126-1047 571.75 ± 355.88**	126-1318[a] 529.67 ± 557.49**	417-1660[a] 1041.33 ± 507.5*

[#]The same results used as control for all the three experimental groups before primary vaccination, [1]Primary vaccination was made at the age of 14 days after blood collection, [2]Booster vaccination was made at the age of 21 days after blood collection, [3]Challenged with BD-3 Wt- A wild-type Bangladeshi field strain of vvIBDV (BD-3/99) (Islam *et al.*, 2001) at the age of 40 days after blood collection, n = No. of samples. [a](n = 3), Significantly differed at *($p < 0.05$) and **($p < 0.01$) levels.

In case of TAD Gumboro vac forte®, 7 days (839 ± 219.34) and 14 days (258 ± 44.80) after primary vaccination, the ELISA antibody titre significantly ($p < 0.01$) decreased but 7 days after booster vaccination, the ELISA antibody titre significantly ($p < 0.01$) increased (1299 ± 37.51). This finding supports the report of Savic *et al.* (1998).

All the control sera revealed significant ($p < 0.01$) decrease of ELISA antibody titre up to day of challenge infection. There was an insignificant increase in ELISA antibody titre 7 days after booster vaccination in case of unvaccinated control groups for TAD Gumboro vac® and TAD Gumboro vac forte® only (Table 4). This might be due to horizontal transmission of booster vaccine virus from the vaccinated groups to the control group.

Table 3. Antibody titre detected by indirect ELISA in broiler chickens immunized against IBD with Bur-706® vaccine (Merial, France)

Vaccine used	Pre-vaccination# (n = 3) Day-old[1]	Post-vaccination (n = 3)				Post-challenge (n = 3) 35 days
		Post-primary		Post-booster		
		7 days	14 days[2]	21 days	28 days[3]	
Bur-706®	4169-6166 4867 ± 919.38	1380-3715 2596 ± 955.69*	1023-1995 1380 ± 436.74**	501-1175 812 ± 277.67*	107-646 343 ± 198.71**	661-1862 1145 ± 517.25**
Control	4169-6166 4867 ± 919.38	2818-3467 3199 ± 276.60*	1995-2951 2395 ± 405.57**	1585-3020 2200 ± 603.50**	288-2344 855 ± 860.78**	891-1820 1215 ± 428.38*

#The same results used as control for all the three experimental groups before primary vaccination, [1]Primary vaccination was made at the age of 1-day-old after blood collection, [1]Booster vaccination was made at the age of 14 days after blood collection, [3]Challenged with BD-3 Wt- A wild-type Bangladeshi field strain of vvIBDV (BD-3/99) (Islam *et al.*, 2001) at the age of 28 days after blood collection, n = No. of serum samples, Significantly differed at *($p < 0.05$) and **($p < 0.01$) levels.

Table 4. Antibody titre detected by indirect ELISA in broiler chickens immunized against IBD with TAD® Gumboro vaccines (Lohmann Animal Health, Germany)

Vaccine	Pre-vaccination (n = 3)# 14 days[1]	Post-vaccination (n = 3)			Post-challenge (n = 3) 42 days
		Post-primary		Post-booster (35 days[3])	
		21 days	28 days[2]		
TAD Gumboro vac®	1995-2951 2395 ± 405.57	776-1072 887 ± 131.68**	269-1175 606 ± 369.41**	269-1230 596 ± 448.61	1585-2089 1921 ± 237.59**
TAD Gumboro vac forte®	1995-2951 2395 ± 405.57	661-1148 839 ± 219.34**	186-309 258 ± 44.80**	1259-1349 1299 ± 37.51**	1259-1905 1583 ± 263.73
Control	1995-2951 2395 ± 405.57	1585-3020 2200 ± 603.50	288-2344 855 ± 860.78**	646-1995 1330 ± 550.89	1445-2570 2195 ± 530.33*

#The same results used as control for all the three experimental groups before primary vaccination, [1]Primary vaccination was made at the age of 14 days after blood collection, [2]Booster vaccination was made at the age of 28 days after blood collection, [3]Challenged with BD-3 Wt- A wild-type Bangladeshi field strain of vvIBDV (BD-3/99) (Islam *et al.*, 2001) at the age of 35 days after blood collection, n = No. of serum samples, Significantly differed at *($p < 0.05$) and **($p < 0.01$) levels.

Table 5. Antibody titre detected by indirect ELISA in broiler chickens immunized against IBD with Bursaplex™ vaccine (Merial Select, Inc., USA)

Vaccine used	Pre-vaccination# (n = 5) Day-old[1]	Post-vaccination (n = 5)			Post-challenge (n = 5)	
		7 days	14 days	21 days[2]	28 days	35 days
Bursaplex	3614.10-5432.50 4229.79±628.89	2191.68-3421.81 2719.27±428.08*	931.55-1832.10 1482.25±372.50**	565.33-1764.46 1079.64±395.01**	594.31-1943.58 1333.09±499.25	2157.74-3810.66 2848.44±589.92*
Control	3614.10-5432.50 4229.79±628.89	2156.17-2600.16 2340.08±151.09*	1059.25-2870.78 1470.43±702.19**	153.82-847.23 477.83±230.49**	220.10-666.81 373.39±157.86	1177.61-2162.72 1774.73±330.50**

#The same results used as control for all the three experimental groups before primary vaccination, [1]Primary vaccination was made at the age of 1-day-old after blood collection, [2]Challenged with BD-3 Wt- A wild-type Bangladeshi field strain of vvIBDV (BD-3/99) (Islam *et al.*, 2001) at the age of 21 days after blood collection, n = No. of serum samples, Significantly differed at *($p < 0.05$) and **($p < 0.01$) levels.

Nobilis® Gumboro D78, TAD Gumboro vac® and Bur-706® vaccinated groups revealed significant ($p < 0.01$) increase of ELISA antibody titre 7 days post-challenge while Nobilis® Gumboro 228E and Bursaplex™ vaccinated groups showed significant ($p < 0.05$) increase of antibody titre after 7 days of challenge (Table 2-5). Insignificant increase of antibody titre was recorded in TAD Gumboro vac forte® vaccinated group (Table 4).

These results suggest that TAD Gumboro vac forte® (Intermediate plus) and Bursaplex™ (Merial Select, Inc. Gainesville, USA) can be used to immunize the broiler birds sufficiently against IBD. However, it is necessary to estimate the optimum vaccination timing, i.e., to determine when maternal antibodies in chicks will decline to levels that the vaccines can overcome.

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