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Comparison of the Immunogenicity and Pathogenicity of a Genetically Engineered Infectious Bursal Disease Virus Vaccine and Two Commercial Live Vaccines in Chickens with Maternal Antibodies

Erfan Ullah Chowdhury[1*], Momota Rani Paul[2], Shukes Chandra Badhy[3] and M. Rafiqul Islam[4]

[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.
[2]Renata Agro Industries Ltd., Plot # 1, Milk Vita Road, Section # 7, Mirpur, Dhaka-1216, Bangladesh.
[3]Department of Livestock Services, Krishi Khamar Sarak, Farmgate, Tejgaon, Dhaka-1215, Bangladesh.
[4]Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author EUC designed the study, collected the samples, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MRI supervised and managed the analyses of the study. Authors MRP and SCB assisted in sample collection, laboratory analyses, literature search, and writing report. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to determine the immunogenicity and pathogenicity of an experimental infectious bursal disease virus (IBDV) live vaccine, BD3-tc, derived by genetic engineering from a Bangladeshi very virulent IBDV strain. Two commercial live IBDV vaccines, D-78 and 228E, were included for comparison. Two-hundred 1-day-old commercial layer chickens

**Corresponding author: E-mail: euc0001@auburn.edu;*

were raised in relative isolation and at 14 days of age the chickens were divided into 4 groups in 4 separate houses. Three groups were vaccinated intraocularly with the BD3-tc or D-78 or 228E at 14 and 21 days of age and the fourth group served as an unvaccinated control. At 21, 28, and 35 days of age, chickens were individually weighed, bled, and necropsied. The bursa of Fabricius (BF) from each chicken was collected, weighed, formalin fixed, and examined histologically. The immunogenicity was evaluated by serum antibody titer to IBDV as measured by an enzyme linked immunosorbent assay. The pathogenicity was analyzed by bursa/body-weight (B/Bw) ratio and gross and histopathological lesions in BF. The chickens were found to have high maternal antibody (mAb) titers (mean titer = 7324 on day 3). Following primary vaccination, no significant level of acquired antibody was observed in any of the vaccine groups. However, on day 35, two weeks after booster, the 228E group had nearly unchanged and the BD3-tc group had a slight increase of antibody titer. In contrast, antibody level in the D-78 group continued to decline. No significant changes in B/Bw ratios and bursal lesion scores were observed in any of the vaccine groups. Together, these findings show that high mAb titers in chicks can interfere with the take of IBDV vaccines, however, the 228E and BD3-tc vaccines are capable of breaking through the mAb at a relatively higher level as compared to the D-78.

Keywords: Infectious bursal disease virus, IBDV; very virulent IBDV; bursa of Fabricius; IBDV vaccine; maternal antibodies; immunogenicity; pathogenicity.

1. INTRODUCTION

Infectious bursal disease (IBD), also called Gumboro disease, is an acute, highly contagious and immunosuppressive viral infection of chickens. The disease is caused by a bisegmented, double stranded RNA virus belonging to the genus *Avibirna* virus and the family Birnaviridae [1,2,3]. Of the two IBD virus (IBDV) serotypes, only serotype 1 is pathogenic and causes disease in chickens of usually 3 to 6 weeks of age [4]. Based on their antigenic variation and virulence, serotype 1 is further classified into four groups: classical virulent, attenuated, antigenic variant, and very virulent (vv) strains [5]. The pathogenic virus infects and lyses immature B-lymphocytes in the bursa of Fabricius (BF) and results profound immunosuppression in infected chickens [6]. The IBDV infection usually causes 100% morbidity, but the mortality varies depending on the strain with the highest mortality, 60-100%, is caused by the vvIBDVs [7].

IBD can only be prevented by vaccination along with strict biosecurity measures. Both inactivated and attenuated live IBDV vaccines are commercially available and widely used in the poultry industry [8]. Inactivated vaccines are mainly given to the breeder or layer flock to confer immunity to the progeny [8]. Live attenuated vaccines are of 3 types: tissue culture adapted highly attenuated or mild vaccine, less attenuated or intermediate vaccine, and chicken's bursa derived intermediate plus or hot vaccine [9]. Although live IBDV vaccines are highly efficacious, the vaccine efficacy decreases in the presence of maternal antibody (mAb) and some of them may cause bursal atrophy [10]. Moreover, sometimes these vaccines cannot prevent outbreaks of IBD in the field due to the antigenic variations in the field isolates. Additionally, there are no vaccines against vvIBDV yet commercially available. This could be due to the fact that the vvIBDV does not normally grow in conventional tissue culture. Whereas, their adaptation in tissue culture by repeated blind passages, first in chicken embryos then in cell culture, make them too much attenuated. Therefore, a highly efficacious and safe IBD vaccine is urgently needed. In recent years, new approaches have been utilized for the development of IBD vaccines, such as, recombinant, subunit, DNA, and more recently genetically engineered IBDV vaccines.

Like many countries in the world, IBDV is one of the leading causes of poultry mortalities in Bangladesh. In 1999, 3 isolates of IBDV were obtained from chickens in Bangladesh and designated as BD1/99, BD2/99 and BD3/99 [11]. Molecular characterization of these isolates demonstrated that they were antigenically and genetically similar to the vvIBDVs reported from Europe, Asia, and Africa [11]. The BD3/99 was genetically engineered through site directed mutagenesis of two amino acids at position 253 (Glutamine to Histidine) and 284 (Alanine to Threonine) in the viral protein 2 (VP2) [12]. Subsequently, this virus was successfully adapted to grow in chicken embryo fibroblast cell culture [12]. The tissue culture (tc) adapted

BD3/99 vvIBDV was termed as BD3-tc and was found to be partially attenuated for commercial chickens [12]. The purpose of the current study was to evaluate the BD3-tc as a potential vaccine candidate in comparison with two commercially available IBDV vaccines - D-78, a tissue culture derived intermediate strain, and 228E, a bursa derived intermediate plus strain. To this end, we evaluated the active immune response by measuring antibody titer in commercial chickens following vaccination with the BD3-tc. We also studied the residual pathogenicity of the BD3-tc in terms of histopathological lesions and atrophy of the BF.

2. MATERIALS AND METHODS

2.1 Experimental Animals, Housing, and Management

Two hundred 1-day-old commercial Brown Nick layer chickens were obtained from a commercial source. The chickens were vaccinated against the Marek's disease in the hatchery. The experiment was conducted in the Department of Pathology at the Faculty of Veterinary Science, Bangladesh Agriculture University, Mymensingh, Bangladesh. The chickens were reared for 5 weeks in well ventilated houses, maintaining strict biosecurity with an *ad libitum* supply of food and water.

2.2 Propagation, Harvesting, and Titration of Experimental Vaccine Virus, BD3-tc

The BD3-tc virus stock, which was previously passaged 5 times in chicken embryo fibroblast (CEF) cell culture, was further propagated in primary CEF cell culture. The primary CEF cell culture was prepared from 9 to 11 days old chicken embryos by warm trypsinization method as described previously [13]. The BD3-tc virus was propagated in the CEF cell sheet grown in 100 cm^2 flasks and the cytopathic effect (CPE) was observed using an inverted microscope. When a maximum CPE was observed, the infected cell culture was frozen and thawed for three times. After the final thawing, the infected culture supernatant was collected, finally divided into small aliquots, and stored at -20°C. The titer of infectious virus present in the culture was determined by plaque assay on CEF cells as described previously [14]. The stock suspension of BD3-tc (passage 6) was found to have 3×10^4 plaque forming units (pfu) / mL.

2.3 Commercial Vaccines

Two IBDV commercial imported live attenuated vaccines were used: Nobilis® Gumboro D78 and Nobilis® Gumboro 228E (Intervet, The Netherlands).

2.4 Field Sera

The field sera used in this experiment were obtained from a replacement parent stock. The chickens were vaccinated with an intermediate IBDV vaccine at 14 days of age and sera were collected at 1, 14, 28, and 42 days of age, 5 samples at every occasion.

2.5 Vaccination and Sampling Procedures

Of the total 200 chicks, 30 chickens were initially used for serum collection on day 3, 7, and 14 by randomly selecting 10 chickens per occasion. On day 14, the remaining 170 birds were divided into 4 groups in 4 different houses. Three groups, each with 40 chickens per group, were intraocularly vaccinated with a single dose of either BD3-tc (30 µl; ~10^3 pfu as determined by titration analysis), or D-78 (36µl, per manufacturer's instruction), or 228E (36µl) at 14 days and boosted at 21 days of age. The fourth group, containing 50 chickens, received only phosphate buffered saline (PBS) on each occasion of vaccination and served as the unvaccinated control. The chickens were monitored daily for any clinical signs after vaccination. On day 21, 7 days after first vaccination, 10 chickens, selected randomly, from each experimental group were euthanized by cervical dislocation, bled by cardiac puncture, individually weighed, and necropsied for the collection and analysis of BF. The same procedures were carried out on 28 and 35 days of age. Gross lesions, if any, observed during necropsy, were recorded. Sera were separated from blood samples and stored at -20°C until used. Bursal tissue samples were fixed in 10% neutral buffered formalin.

2.6 Serology

Serum samples obtained on day 3, 7, 14, 21, 28, and 35 days of age were analyzed for the presence of anti-IBDV by an indirect enzyme linked immunosorbent assay (ELISA) using a commercial kit (IBD test kit, IDEXX laboratory, Inc., Westbrook, Maine 04092, USA). The ELISA was performed according to the manufacturer's

instruction. The absorbance values were determined at 650 nm using a spectrophotometer (SpectraMax® 340PC384 Microplate Reader, Molecular Devices Inc., USA). The titer was calculated from the absorbance value using the formula supplied with the ELISA kit.

2.7 Gross and Microscopic Analysis of Bursa of Fabricius

Each BF was weighed and bursa-body weight (B/Bw) ratio was calculated by dividing the bursa weight for the body weight multiplied by 1000. Formalin fixed BF tissues were submitted to histopathological examination to determine the presence of lesions. The slides were studied by a pathologist and the bursal lesions were scored on a 0 to 4 scale. The criteria for scoring lesions were: 0 equaled BF with apparently normal follicle; +1 had a mild lymphoid depletion; +2 had a moderate lymphoid depletion; +3 had a severe lymphoid depletion; and +4 had a severe lymphoid depletion with marked follicular atrophy and with or without cystic spaces.

2.8 Statistical Analysis

All results are expressed as mean ± standard error of mean (SEM). Statistical analyses were performed using the Microsoft Excel program. The variation in different groups were determined by Student t test.

3. RESULTS AND DISCUSSION

At 3 days of age, chicks were found to have very high levels of mAb (mean ELISA titer=7324±1805) which gradually declined over the time (Fig. 1A). Subsequently, during the first vaccination on day 14 the mean titer was 3277±868 and even at the age of 35 days the control group had quite high and positive antibody titer (671±293) (Fig. 1A). Following vaccination at day 14 and 21, the level of antibody titer continued to fall in all groups until day 28 (Fig. 1A). At 35 days of age, the titer in the D-78 group dropped further, but remain nearly unchanged in the 228E group, and increased slightly in the BD3-tc group. The antibody titers in the BD3-tc (1866±907) and 228E (1303±1037) groups at 35 days were significantly higher than that in the control group (365±293; p=0.05) (Fig. 1A).

The failure of all three vaccine to induce remarkable active immunity might be due to the high mAb titers during vaccination. To test this hypothesis, field sera from a replacement parent stock were examined. Interestingly, these chickens had a low level of mAb at day 1 (1005±364) (Fig. 1B), which dropped to 746±242 at the time of vaccination (day 14). So, even a mild commercial vaccine (D78) was well taken and the antibody titer gradually increased following vaccination (Fig. 1B).

The findings of this study demonstrated that a high level of mAb during primary immunization interfered with the take of IBDV vaccines. Similar finding was observed previously by Solano et al. [15]. In their study, when high mAb (ELISA titer 16,384 on day 1) bearing chickens were vaccinated at 1 or 15 days of age with an intermediate live IBDV vaccine (Bursine-2), no detectable immediate primary antibody response was observed in chickens. Similarly, Rautenschlein et al. [16] demonstrated that at an average virus-neutralizing mAb of 1782 during vaccination in commercial broilers, only the intermediate plus vaccine was able to induce a significant level of IBDV antibodies after 18 days, while the intermediate vaccines did not. In contrast, an average mAb titer of 104 at the day of vaccination, both vaccines, intermediate and intermediate plus, induced circulating antibodies. Alam et al. [17] performed a prime-boost IBDV immunization experiment in broilers with a live IBDV vaccine. They observed that a primary vaccination in broilers at 14 days with a mean ELISA mAb titer of 772 resulted in a minimal increase of titer (mean=1076) after 7 days. However, a subsequent booster at day 21 markedly increased the titer (mean= 1757 at 28 days). In our study, the ELISA mAb titer during primary immunization was very high (3277±868) which completely neutralized viruses in all three vaccines. Notably, the mAb titer was also very high during booster vaccination at day 21 as indicated by the presence of high antibody titer in the control group (average ELISA titer=1961). This suggested that even after booster vaccination, the vaccine viruses were under continual neutralization effect by mAB. This neutralization effect of mAb was evident by continual decline of titer in the D-78 group after booster. However, there was a relatively unchanged and an increasing antibody titer on day 35, two weeks after the booster, in the 228E and BD3-tc vaccine groups, respectively. This finding suggested that the 228E and BD3-tc vaccines were able to breakthrough the neutralization effect of the mAb following the booster on day 21.

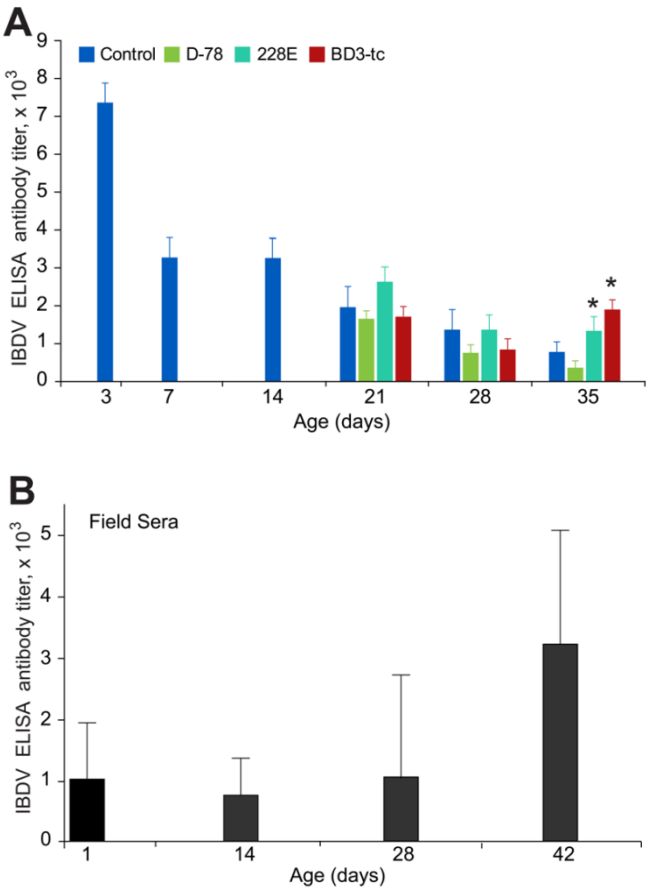

Fig. 1. IBDV antibody titer in the experimental and field chickens at different time points. (A) Antibody titer in control and immunized (D-78, 228E, and BD3-tc) groups of experimental chickens. Birds were vaccinated at 14 & 21 days of age. Serum samples were collected at 3, 7, and 14, 21, 28 and 35 days of age and tested for IBDV by ELISA. On day 35, antibody titer in 228E and BD3-tc was significantly higher than the control). Results are expressed as mean ± SEM (n=5 chickens/group) and compared by using Student t test. *P=0.05 compared with unvaccinated control. (B) Antibody titer of field sera from a replacement parent stock. Chickens were vaccinated at 14 days of age, and sera were collected at day 1, 14, 28, and 42. Results are expressed as mean ± SEM (n=5 chickens/occasion)

In the study of residual pathogenicity, no characteristic gross lesions were observed in any of the vaccine groups. However, at 28 days of age mild hemorrhage in BF was observed in one bird in the 228E group, and at 35 days mild hemorrhages in thigh muscle were found in most of the necropsied chickens in D-78 and 228E groups (data are not shown here). The B/Bw ratios of chickens were determined at 21, 28, and 35 days and no significant bursal atrophy was observed (Fig. 2). In all occasions, no significant differences in B/Bw ratios were observed between chickens in vaccine groups and control chickens (Fig. 2).

The histopathological lesions in vaccinated chickens were mainly characterized by mild to moderate lymphoid depletion of the follicles in BF with the maximum bursal lesion score was 2 (Table 1). No significant variation in the lesion scores was found in three vaccinated groups. No detectable lesions were observed in the control group (lesion score 0; Table 1).

B/Bw ratio is one of the most important parameters to evaluate residual pathogenicity of IBDV vaccines [18]. In general, a hot IBDV vaccine, such as an intermediate plus vaccine, 228E, is capable to destroy B-lymphocytes present on the BF, reduces their size and therefore causes considerable reduction in B/Bw ratio than a mild IBDV live vaccine [19]. However, in the present study, no significant changes in the B/Bw ratios and minimal

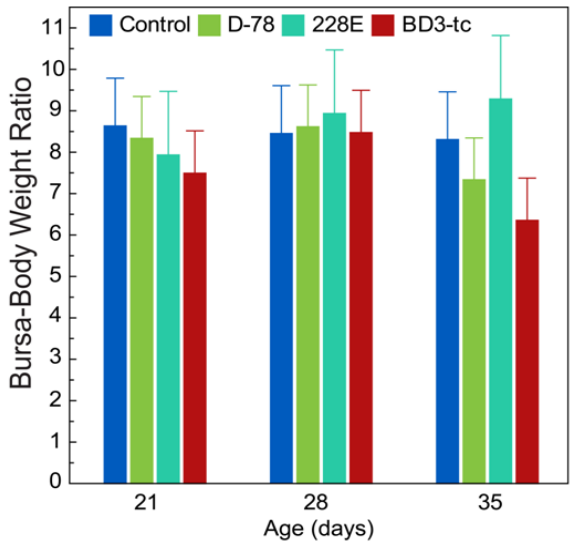

Fig. 2. Bursa-body weight (B/Bw) ratios in experimental chickens at different time points after vaccination. Chickens were vaccinated at day 14 & 21 days of age. Results are expressed as mean ± SEM (n=3 chickens/group) and compared by using Student t test

Table 1. Quantitative histological analysis of bursa of Fabricius expressed in lesion scores of the experimental groups after vaccination

Age (Days)	Bursal lesion score of individual bird (n=3 chickens/group)			
	Experimental groups			
	Control	D-78	228E	BD3-tc
21	0, 0, 0	1, 2, 2	1, 2, 1	1, 1, 1
28	0, 0, 0	1, 2, 1	1, 2, 1	0, 2, 1
35	0, 0, 0	1, 2, 2	1, 2, 2	1, 1, 1

histopathological lesion scores in BF in all vaccine groups including 228E indicated that the vaccine virus replicated only partially in the BF. These findings again suggested that high mAb titers in chickens during vaccination neutralized the vaccine viruses. A similar finding was reported previously by Horner et al. [20]. In their study they found that chickens with high mAb titers showed no serologic response, almost no histopathological changes (lesion score was 0 in most cases) and minimal changes in B/Bw ratio following vaccination with several live vaccines at 14 days of age.

4. CONCLUSION

Due to the presence of high mAb in chickens, the relative immunogenicity and residual pathogenicity of the three vaccines could not be truly evaluated. However, considering the presence of significantly higher antibody titer on day 35 in 228E and BD3-tc group than in the control group, these two vaccines can induce better active immune response in the presence of mAb. Taken together, our findings suggested that the immunogenicity and residual pathogenicity of BD3-tc are comparable to that of the intermediate plus vaccine, 228E. However, for a better evaluation of the BD3-tc as a potential vaccine candidate against IBDV in chickens, the parameters of this study should be re-investigated in a specific pathogen free chicken model. Future Studies should also be directed toward evaluation of the protective efficacy of this vaccine candidate against vvIBDV challenge in chickens.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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