# Comparison of Pathogenicity and Serologic Response of Four Commercial Infectious Bursal Disease Live Vaccines

***Short Communication***

**Hedayati[*1], A., Nili, H.,[2] Bahonar, A.[3]**

*1. Poultry Diseases Dept., Iranian Veterinary Organization, Iran*

*2. Clinical Sciences Dept., School of Veterinary Medicine, University of Shiraz, Iran*

*3. Food Hygiene Dept., Faculty of Veterinary Medicine, University of Tehran, Iran*

Received ; accepted

***Summary***

Various commercial vaccines for immunization of broiler chickens against infectious bursal disease (IBD) are available, so it would be appropriate to compare the pathogenicity and immune response of chickens to these vaccines. In this study the pathogenicity and serologic response of four IBD vaccines, cloned D78®, Bursine-2®, Bursimune® and Cevac Gambo-L®, were evaluated in specific pathogen free (SPF) chickens. 100 SPF chicken were divided into five equal groups (one control group and four vaccinated groups) kept in isolator units and vaccinated at 16-day-old via the eye-drop route. At 5, 10 and 20 day post vaccination birds from each group were weighted, bled, and then necropsied. Lesions were recorded and the bursa of Fabricius was taken out, weighted and was fixed in 10% neutral buffered formalin and processed for histopathological examination. The pathogenicity and serologic effects of the IBD vaccines were evaluated by the antibody response, the bursa, spleen and thymus/body weight ratios and histopathological lesions of the bursa. No any clinical signs and mortality was observed in all groups. The results of this study indicate that D78 and Gambo-L vaccines showed to be more pathogenic and caused more severe bursal damages and also induced higher ELISA titers in serological evaluation.

***Key words:*** infectious bursal disease vaccine, pathogenicity, immune response, ELISA

* Author for correspondence. E-mail:afshin221@yahoo.com

***Introduction***

Infection bursal disease is one of the most important viral infections occurring in young chickens. The disease is caused by Birnaviridae family designated as infectious bursal disease virus (IBDV) (Dobos *et al* 1979). Two serotypes (serotype 1 and 2) have been recognized that naturally infect chickens. However, only IBD strains belonging to serotype 1 is considered to be pathogenic (McFerran *et al* 1980, Jackwood & Saif 1983, Jackwood *et al* 1985). IBDV is a lymphotropic pathogen with a special predilection for differentiating cells in the bursa of Fabricius. Infection can induce B-cell apoptosis, necrosis, and bursal atrophy with a concomitant suppression of the humoral response (Sivanandan & Maheswaran, 1980, Muller 1986). Damage to the bursa may occur with a severe inflammatory response such as the one described for standard IBDV strains (Benton *et al* 1967, Tanimura *et al* 1995). However, atrophy of this organ may be induced with little or no inflammation (Allan *et al* 1972, Faragher *et al* 1974, Tanimura *et al* 1995). Successful vaccination practice and strategy necessitates proper selection of vaccine and good vaccination plan in broiler chicken. Despite extensive use of live IBD vaccines in Iranian broiler industry, IBD still causes great economic losses and has been incriminated in many incidences of high mortality in the industry. Giambron and Clay (1986) compared four commercial live IBD vaccines for their immunogenicity, stability and pathogenicity and concluded that more intermediate vaccines seems to be the vaccines of choice in commercial chicken flocks since they are more efficacious. Rautenschlein *et al* (2003) compared immunopathogenesis of mild, intermediate and virulent strains of classic IBDVs and showed that the most virulent strain induced the most prominent bursal damage and significant suppression of the mitogenic response and the mild vaccines induced no detectable lesions in the bursa. Objectives of this study were to determine the immunogenicity and the pathogenecity of four commercially available live intermediate vaccines administrated at 16-day-old SPF chickens.

## *Materials and Methods*

**Chicken.** 100 SPF emberionated eggs obtained from a Lohmann Animal Health flock were used. The eggs were incubated, and hatched chicks were kept in modified Montair HM-1500 Isolator units (placed in Central Veterinary Laboratory) maintained with filtered air under negative pressure. Birds were received a sterile corn-soybean starter ration.

**IBD Vaccines.** The following four commercial vaccines Bursine-2® (Lukert, Fort Dodge) and Clone Vac® D78 (Clone D78, Intervet) with embryonated egg origin, and Cevac Gambo-L® (L IBDV, Cevac) and Bursimune® (Lukert, Biomune) with cell culture origin were given by eye-drop at 16-day of age. All of them are considered as intermediate live vaccines and administered at the doses recommended by the manufacturer.

**Experimental design**. Chickens were divided into five groups of 20 birds and transferred to HM-1500 isolation units that contained HEPA filters in the intake and exhaust outlets (Montair-Andersen, B.V.). Four groups of 16 day-old SPF chickens were inoculated via the eye-drop routes with one dose of D78, Bursine-2, Bursimune and Gambo-L vaccines. Chickens in the fifth group were kept as uninoculated control. At 5 and 10 days post vaccination (PV) five birds from each group were weighed, bled and euthanatized, then necropsied. Sera were collected and stored at -20C until used to antibody detection. The bursa, spleen and thymus were removed and weighed before being fixed for histopathologic observation. Bursa, spleen and thymus/body weight ratios were calculated for each bird and expressed as arithmetic means in each group of birds by the following formula: organ weight in grams divided by body weight of individual bird in grams multiplied by 1000. At 20 days post vaccination, all remaining birds were treated as above.

**Histopathology**. Bursa was fixed in 10% neutral buffered formalin. Sections were made and stained with heamatoxylin-eosin following conventional procedures. All

lesions were scored from 1 to 4 according to the index defined by Mazariegos *et al* (1990).

**Antibody detection.** To detect the antibody directed against IBDV a commercial ELISA kit, IDEXX FlockChek standard (IDEXX Corporation, Westbrook, ME, USA) for the detection of antibodies directed against IBDV was used. The procedure was conducted as described by manufacturer instruction. The test kits use a 1:500 standard dilution of test serum.

**Statistical analysis.** The average bursa, spleen and thymus/body weight ratios, bursal lesions and ELISA titers were compared with those of control groups using an analysis of variance "ANOVA" (Bonfferony and Chi-Square) followed by SPSS software ($P<0.05$).

## *Results and Discussion*

Results of the pathogenecity experiments are summarized in table 1.

Table 1. *Results of bursal, spleen and thymus/BW ratios following vaccination of SPF chicken with commercial IBDV vaccines*

| Group | Bursal/BW | | | Spleen/BW | | | Thymus/BW | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 d PV | 10 d PV | 20 d PV | 5 d PV | 10 d PV | 20 d PV | 5 d PV | 10 d PV | 20 d PV |
| Bursin-2 vaccine | $3.4^{b}$ ±.79 | $3.28^{b}$ ±.86 | $3.23^{b}$ ±.91 | $3.02^{b}$ ±.37 | $1.13^{a}$ ±.31 | $1.51^{a}$ ±.27 | $3.31^{a}$ ±9.5 | $3.08^{b}$ ±.31 | $4.11^{b}$ ±.26 |
| Bursimune vaccine | $5.68^{a}$ ±.53 | $5.27^{a}$ ±.74 | $6.26^{a}$ ±1 | $1.55^{a}$ ±.33 | $1.53^{a}$ ±.31 | $1.83^{b}$ ±.26 | $3.74^{a}$ ±.33 | $4.37^{a}$ ±.35 | $4.91^{a}$ ±.58 |
| D78 vaccine | $5.33^{a}$ ±.72 | $2.98^{b}$ ±1 | $2.58^{b}$ ±1 | $3.01^{b}$ ±.22 | $1.87^{a}$ ±.26 | $1.76^{a}$ ±.25 | $3.29^{a}$ ±.68 | $3.87^{b}$ ±.42 | $3.28^{b}$ ±.4 |
| Gambo-L vaccine | $4.8^{a}$ ±1.2 | $2.4^{b}$ ±.56 | $2.84^{b}$ ±1.27 | $3.37^{b}$ ±.64 | $2.09^{a}$ ±.69 | $1.73^{a}$ ±.31 | $3.29^{a}$ ±.30 | $4.04^{b}$ ±.42 | $3.47^{b}$ ±.72 |
| Control | $5.15^{a}$ ±.12 | $5.53^{a}$ ±.25 | $5.65^{a}$ ±.20 | $1.28^{a}$ ±.36 | $1.27^{a}$ ±.13 | $1.4^{a}$ ±.22 | $3.76^{a}$ ±.65 | $4.83^{a}$ ±.41 | $5.1^{a}$ ±.12 |

Different lowercase superscripts letters within each column indicate significant differences between groups (chi-square test and one-way ANOVA with Bonfferony test, $P<0.05$). PV =Post vaccination. BW=Body Weight±SD

No clinical signs and mortality were noticed in vaccinated chickens; however necropsy findings showed gelatinous bursa in 2 out of 5 vaccinated chickens in Gambo-L group. Also chickens vaccinated with Bursimune showed bursal swelling 20 days PV.

Histopathological findings showed more severe tissue reaction in the bursa of Fabricius and the spleen of chickens vaccinated with Gambo-L and D78 vaccines. These were including hyperemia, haemorrhage and oedema in more acute stages (5 days PV) followed by bursal depletion, atrophy and cyst formation (10 days PV). In late stage of bursal reaction more connective tissue was present and regenerated bursal follicle were abundant. Chickens vaccinated with Bursine-2 showed very slight to moderate tissue reaction. Five days PV there were slight hyperemia and bursal oedema followed by moderate lymphocyte depletion at 10 and 20 days PV. In chicken vaccinated with Bursimune, moderate to severe bursal damages were observed at 5 days PV including lymphocyte depletion especially in the medulla of the bursal follicles, followed by regeneration at 10 and 20 days PV.

*Bursal score.* The severity of bursal damages which is refered to as bursal score is presented in table 2. Chickens vaccinated with D78 and Gambo-L had highest bursal score respectively.

Table 2. *Necropsy and histopathological lesions in the bursa of Fabricius after inoculation with four commercial IBD vaccines*

| **Group** | **Vaccines Bursal Score** | | |
|---|---|---|---|
| | **5 d PV** | **10 d PV** | **20 d PV** |
| Bursin-2 vaccine | 1±0 | 1.2±.44 | 1.5±.47 |
| Bursimune vaccine | 1±0 | 1±0 | 1±0 |
| D78 vaccine | 2.1±.54 | 1.2±.24 | 2.4±44 |
| Gambo-L vaccine | 2.4±.24 | 1.6±.58 | 1.8±.44 |
| Control | 1±0 | 1±0 | 1±0 |

Bursal lesion score: 1=no lesions, 2=mild cell depletion in a few follicles, 3=moderate atrophy or cell depletion in 1/3 to 1/2 of the follicles. 4=severe necrosis and atrophy in all follicles, PV= Post Vaccination and ±SD

*ELISA.* The results of antibody responses following vaccination with D78, Gambo-L, Bursine-2 and Bursimune are showed in table 3. As indicated in the table, at 5 days PV all vaccinated chickens showed very low level of antibody responses. But at 10 and 20 days PV there was significant seroconversion in D78 and Gambo-L groups. However in Bursine-2 and Bursimune antibody responces was much lower compared to Gambo-L and D78 vaccinated groups ($P<0.05$).

Table 3. *Antibody responses measured by indirect commercial ELISA kit (IDEXX) following vaccination of SPF chickens with four available IBD vaccines*

| Group | ELISA titer | | |
|---|---|---|---|
| | 5 d PV | 10 d PV | 20 d PV |
| Bursin-2 vaccine | 1±4 | 79±97 | 571±365 |
| Bursimune vaccine | 1±0 | 1±0 | 267±478 |
| D78 vaccine | 75±65 | 968±514 | 3255±989 |
| Gambo-L vaccine | 50±106 | 663±194 | 2236±723 |
| Control | 0±0 | 0±0 | 0±0 |

Study of the pathogenecity of four commercial IBD vaccines showed considerable variation in their pathogenicity. According to the results obtained in the present study vaccine D78 and Gambo-L proved to be more pathogenic than Bursine-2 and Bursimune. This was supported by bursal and thymus/BW ratios reduction and bursal score. Similar results reported by Giambron and Clay (1986) regarding to D78 pathogenecity. Although it has been reported that classical IBD viruses propagated in cell culture show lower pathogenecity than embryonated egg propagated viruses (Chavez *et al* 2002, Hassan & Saif 1996), the results of this study show that the Gambo-L Vaccine which is a cell culture propagated one, showed more pathogenecity than Bursine-2 which is embryonated egg origin.

Mazariegos *et al* (1990) showed that intermediate vaccines varied in their pathogenecity. Based on bursal damage, bursal/BW ratios and histopathological findings, they divided intermediate vaccines into 3 pathogenic categories "highly

pathogenic, moderate pathogenic and low pathogenic or mild". According to this results D78 and Gambo-L vaccines showed to be moderate pathogenic and Bursimune was low pathogenic or mild. Since D78 and Gambo-L showed to be more pathogenic and can cause severe bursal damage, it is not recommended to use them before 7 days of age. The results of antibody response using ELISA kit support pathogenecity findings. Since more pathogenic vaccine such as D78 and Gambo-L induced higher ELISA antibody titer. This finding was supported by Rautenschlein (2003), Giambron and Closser (1990) and Chavez *et al* (2002).

In conclusion, D78 and Gambo-L vaccines which can cause more bursal damage and ELISA antibody titer should be used only in chickens with relatively high or moderate maternal immunity to reduce the adverse effects of these vaccines (Hassan & Saif 1996, Mazariegos *et al* 1990).

### *Refferences*

Allan, W.H., Faraghar, J.T. and Cullen, G.A. (1972). Immunosuppression by the infectious bursal agent in chicken immunized against Newcastle disease. *The Veterinary Record* 90:511-512.

Benton, W.J., Cover, M.S. and Rosenberger, J.K. (1967). Studied on the transmission of the infectious bursal agent of chickens. *Avian Diseases* 11:430-438.

Chavez, I.R., Rosenberger, J.K., Cloud, S.S. and Pope, C.R. (2002). Characterization of the antigenic, immunogenic, and pathogenic variation of infection bursal disease virus due to propagation in different host systems (bursa, embryo, and cell culture). *Avian Pathology* 31:458-492.

Dobos, P., Hill, B.J., Hallet, R., Kells, D.T.C., Becht, H. and Teninges, D. (1979). Biophysical and biochemical characterization of five animal viruses with bisegmented double-stranded genomes. *Journal of Virology* 32:593-605.

Faragher, J.T., Alla, W.H. and Wyeth, C.J. (1974). Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease. *The Veterinary Record* 95:385-388.

Giambrone, J.J., Clay, R.P. (1986). Evaluation of the immunogenecity, stability, pathogenicity, and immunodepressive potential of four commercial live infection bursal disease vaccines. *Poultry Science* 65:1287-1290.

Giambron, J.J., Closser, J. (1990). Efficacy of live vaccines against serologic subtypes of infectious bursal disease virus. *Avian Diseases* 34:7-11.

Hassan, M.K., Saif, Y.M. (1996). Influence of the host system on the pathogenicity, immunugenicity of infectious bursal disease virus. *Avian Diseases* 40:553-561.

Jackwood, D.J., Saif, Y.M. (1983). Prevalence of antibodies to infectious bursal disease virus serotypes 1and2 in 75 Ohio chicken flocks. *Avian Diseases* 27:850-854.

Jackwood, D.J., Saif, Y.M. and Moorhead, P.D. (1985). Immunogenicity and antigenicity of infectious bursal disease virus serotype 1and2 in chickens. *Avian Diseases* 29: 1184-1194.

Mazariegos, L.A., Lukert, P.D. and Brown, J. (1990). Pathogenicity and immunosuppressive properties of infectious bursal disease "Intermediate" strains (1990). *Avian Diseases* 34:203-208.

McFerran, J.B., Mcnulty, M.S., McKillop, E.R., Conner, T.J., McKracken, R.M., Collins, D.S. and Allan, G.M. (1980). Isolation and serologic studies with infectious bursal disease virus from fowl, turkey and ducks: Demonstration of a second serotype. *Avian Pathology* 9:395-405.

Muller, H. (1986). Replication of infectious bursal disease virus in lymphoid cells. *Archives of Virology* 9:395-405.

Rautenschlein, S., Yeh, H.Y. and Sharma, J.M. (2003). Comparative immunopathogenesis of mild, intermediate, and virulent strains of classic infectious bursal disease virus. *Avian Diseases* 47:66-78.

Sivanandan, V., Meheswaran, S.K. (1980). Immune profile of infectious bursal disease. 1. Effect on infectious bursal disease virus on peripheral blood T and B lymphocytes in chickens. *Avian Diseases* 24:715-725.

Tanimura, N., Tsukamoto, K., Nakamura, K., Narita, M. and Maeda, M. (1995). Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunochemistry. *Avian Disease*s 39:9-20.