Pakistan J. Zool., vol. 46(4), pp. 959-966, 2014.

Comparative Study of Commercially Available Infectious Bursal Disease Vaccine with Egg Attenuated Live Vaccine

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Abstract.- Infectious bursal disease (IBD) is one of the most contagious viral and immunosuppressive diseases in young chickens. Vaccination at proper time is the only way to control the disease. In Pakistan, however, the development of an efficacious and economical vaccine is a major issue. The objective of the study was, therefore, to develop an effectual and inexpensive vaccine. In total, 300 birds were divided into six groups (A to F), each of 50 birds and inoculated subcutaneously on 10th day of age. Three different passaged virus (8, 16 and 24), field isolate and commercial IBD vaccine were tested. The gross pathological and histopathological changes were recorded for bursa, thymus, muscles and kidney. The serum samples were collected for antibody titration against IBDV. Significant changes including swelling and hemorrhages started at 48th h to 10th day post inoculation (PI) in group receiving commercial vaccine, whereas group with inoculation schedule of 24th passage showed very low bursal lesion with regards to the control group. Greater bursa body weight ratio was observed in the group receiving commercial vaccine compared with the one units field isolate and the one units 8th passage, whereas it decreased in groups with 16th and 24th passage. The titre of virus was high in the groups with field isolates and that of 8th passage, whereas group with commercial vaccine and 24th passage had moderate titre with respect to other groups. Bursal changes were minimal in all groups except for group with 24th passage, where no changes were found at 24 h PI relative control group. There were no histopathological changes in the muscles, kidney and thymus in the 24th passage group E respect to all other groups. Greater antibody titer and highest (100%) protection against disease was observed in this group with regard to other groups. On the basis of our results, 24th passage virus can be used to protect the birds from IBD.

Keywords: Infectious bursal disease, immunosuppressive, egg attenuated, live vaccine.

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious acute viral and immunosuppressive disease in young chickens (Rauf, 2011). In Pakistan, viral diseases cause losses of billions of rupees in poultry industry (Waheed *et al.*, 2013). The infectious bursal disease virus (IBDV) serotype 1 targets the lymphoid cells in the bursa as these cells are highly susceptible to virus at their maximum development between three to six weeks of age. This infection leads to the destruction of bursa, the main feature of IBD pathogenesis, the precursor of antibody-producing B cells in the body (Cheville, 1967; Wyeth *et al.*, 1981). The classical forms of the disease outbreak may result in 50% mortality and in broilers; it may exceed 3% in three to six week of age (Müller *et al.*, 2003).

The IBDV, additionally, is a highly immunosuppressive and this immuno-suppression is the main end result of IBD. The other avian species such as turkeys, ducks, guinea fowl and ostriches may be infected, clinical signs, however, occurs solely in chickens (Wyeth *et al.*, 1981). The main clinical signs include watery diarrhoea, depression, ruffled feathers, anorexia, trembling, prostration and death after two to three days of clinical signs onset (Chansiripornchai and Sasipreeyajan, 2005). The major post-mortem lesions may include dehydration of the muscles with numerous ecchymotic hemorrhages, swelling and discoloration of the kidneys, with urates in the tubules, inflammation, edema and bursal hemorrhages or atrophy (Chansiripornchai and Sasipreeyajan, 2009).

At present there are two methods of preventing IBD damage to the immune system of young chicken, which could be used commercially. Birds can be protected by vaccinating the parent

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0030-9923/2014/0004-0959 $ 8.00/0

stock with IBDV (passive protection) or by immunizing the young birds themselves with nonpathogenic isolate. The IBDV is a stable virus, therefore, its control by sanitation and isolation is not possible (Benton *et al.*, 1967). The principal method, therefore, to control this is by vaccination at proper time (Chansiripornchai and Sasipreeyajan, 2009) achieved through the administration of live or killed vaccines. Commercial vaccines against IBD available in Pakistan are imported. Antigenic variation has been observed between local field isolates and imported IBD vaccines virus. In Pakistan, a huge amount of money is being spent for the import of these vaccines. The disease outbreak, however, even occur in spite of vaccination due to their antigenic and immunosuppressive effects. The need exists for infectious bursal disease vaccine, low in virulence that can be applied by a mass vaccination procedure. Keeping in view the tremendous import of IBD vaccine in poultry industry, and to save precious foreign exchange, the present study was, therefore, designated to prepare a safe and efficacious vaccine to control the drastic effects of IBDV. Such a vaccine may minimize immunosuppression and also immunized young chicks possessing passively conferred IBD immunity.

MATERIALS AND METHODS

Pathogenesis of different passaged attenuated infectious bursal disease

Three different passaged virus (eight, 16 and 24), field isolate, and commercial IBD vaccine were tested for pathogenesis for gross pathology and histopathological changes. Humoral response was detected by using IHA. In total, 300 birds were divided into six groups, each of 50 birds and inoculated subcutaneously at 10^{th} day of age, with commercial vaccine (group A), field isolate (group B), 8^{th} passage (group C), 16^{th} passage (group D), 24^{th} passage (group E), and virus (group E). The 6^{th} group was kept as control (group F).

After inoculation, the birds were examined daily for clinical signs up to 14^{th} day. Five birds from each group were slaughtered after every 24, 48, 72 and 96 h, sixth, eighth and 10^{th} day of post inoculation (PI). The gross pathological and histopathological changes were recorded in bursa of fabricious, thymus, kidney, and muscles. The serum samples were collected from five birds of each group at the same day for antibody titration against IBDV. The bursal lesion, thereafter, were scored, with slight modification, according to the scoring system suggested by Singh and Dhawedkar (1997). The lesions were scored on the basis of lymphoid necrosis and scoring was done from 0 to five scales as less than 5% lymphoid follicle affected or no change: 0-5%, no change (NC); 5-25%, minimal (Mn); 26-50%, slight (Sl); 51-75%, mild (Ml); 76-80%, moderate (Md); > 80%, Severe (Sv).

Organ to body weight ratio

Actual weights (g) of the organs (bursa and thymus) were recorded within half an hour after slaughtering. Since organ weight is directly related to body weight, percent weight of these organs to the body weight was also calculated to create uniformity. Organ index was calculated according to Singh *et al*. (1994):

$$\text{Organ index} = \frac{\text{Organ weight (g)}}{\text{Live body weight of bird (g)}} \times 1000$$

Histopathological technique

The bursa, muscles (thigh and breast), thymus and kidneys, were labeled and preserved in 10% buffer formalin for at least 24 to 48 h. for histopathological studies. The samples were processed according to the procedure followed by Dohms and Jaeger (1988). In challenge protection trial, remaining birds were challenged with very virulent strain of the virus.

A Duncan's multiple range test was used to compare the efficacy of five different passages (8, 12, 16, 20 and 24th), commercial vaccine and field isolates against the drastic effects of IBDV.

RESULTS

Gross pathological changes

In bursa, no significant changes were observed after 24 h. PI in groups A and E inoculated with commercial vaccine and 24^{th} passage, respectively, whereas the bursa was slightly changed in groups B, C and D relative to control

Table I.- Gross pathological changes in the bursa of fabricious, muscle, thymus, kidneys of birds (?) inoculated with different infectious bursal disease virus.

Groups/days	1	2	3	4	6	8	10
Bursa of fabricious							
A (Commercial vaccine)	Nc	Mn	Ml	Ml	Md	Ml	Ml
B (field isolates)	Mn	Sl	Ml	Md	Sv	Sv	Md
C (8^{th} passage)	Mn	Sl	Ml	Md	Sv	Sv	Md
D (16^{th} passage)	Mn	Sl	Ml	Ml	Md	Ml	Sl
E (24^{th} passage)	Nc	Nc	Mn	Nc	Nc	Nc	Nc
F (Control)	Nc	Nc	Nc	Nc	Nc	Nc	Nc
Muscle							
A (Commercial vaccine)	Mn	Sl	Ml	Md	Md	Ml	Sl
B (field isolates)	Mn	Sl	Md	Sv	Sv	Sv	Ml
C (8^{th} passage)	Mn	Sl	Ml	Sv	Sv	Sv	M
D (16^{th} passage)	Mn	Sl	Sl	Ml	Md	Md	Ml
E (24^{th} passage)	Nc	Nc	Nc	Nc	Nc	Nc	Nc
F (Control)	Nc	Nc	Nc	Nc	Nc	Nc	Nc
Thymus							
A (Commercial vaccine)	Nc	Mn	Sl	Ml	Ml	Ml	Sl
B (field isolates)	Nc	Mn	Sl	Md	Sv	Sv	Ml
C (8^{th} passage)	Nc	Mn	Sl	Ml	Md	Sv	Ml
D (16^{th} passage)	Nc	Mn	Mn	Sl	Ml	Ml	Sl
E (24^{th} passage)	Nc	Nc	Nc	Nc	Nc	Nc	Nc
F (Control)	Nc	Nc	Nc	Nc	Nc	Nc	Nc
Kidneys							
A (Commercial vaccine)	Nc	Nc	Mn	Sl	Ml	Md	Ml
B (field isolates)	Nc	Mn	Mn	Ml	Md	Sv	Sv
C (8^{th} passage)	Nc	Mn	Mn	Ml	Md	Sv	Md
D (16^{th} passage)	Nc	Nc	Mn	Sl	Ml	Md	Ml
E (24^{th} passage)	Nc	Nc	Nc	Nc	Nc	Nc	Nc
F (Control)	Nc	Nc	Nc	Nc	Nc	Nc	Nc

0-5%, no change (NC); 5-25%, minimal (Mn); 26-50%, slight (Sl); 51-75%, mild (Ml); 76-80%, moderate (Md); > 80%, Severe (Sv).

group F. These changes started at 48^{th} h to 10^{th} day PI in groups A and E compared with the control group. These changes included enlargement and hemorrhages. The groups A and D inoculated with commercial vaccine and 16^{th} passage of virus showed mild lesions, whereas groups B and C inoculated with field isolate and passaged virus 8^{th} produced similar results (severe hemorrhages). The lesion scoring of each organ observed during the study was done by the criteria shown in Table I. The group E inoculated with 24^{th} passage of virus, showed very low bursal lesions with respect to control group F (Table I).

In the muscles, hemorrhages started at 24 h PI to the 10^{th} day in the groups A to D inoculated with commercial vaccine, field isolate and 8, 12, and 16^{th} passages of virus. The intensity of hemorrhages was very high and persisted up to 10^{th} day PI (Table I). In thymus, there were no significant changes in vaccine, field isolate and passaged virus groups (A to E) at 24 h PI compared with the control group. The hemorrhages and enlargement started at 48^{th} h PI in groups A to C, whereas groups A and D showed results comparable to the control group. At 72 h to 10^{th} day PI, the changes were similar in groups A to C while group E showed very mild changes relative to the control group (Table I).

In kidneys, no significant changes were observed in the vaccine, field isolate and passaged virus groups (group A to E) compared with the

control group (F) at 24th h PI. The changes started at 48th h to day 10 PI in groups A to D. The kidneys of these groups were congested and the intensity of changes was significantly greater. The group E, however, showed similar results with regards to control group (Table I).

Organ body weight ratio

Bursa body weight ratio of birds inoculation with different passages of egg adapted IBD virus and vaccine strain indicated significant difference (*P < 0.01*) among various groups and various time intervals with a significance (*P < 0.01*) interaction of both factors. Data showed that groups E and F differ significantly from the rest of the groups. The group A had greater bursa body weight ratio compared with groups B and C. This ratio increased at day 6th and 8 PI, whereas it decreased gradually in groups D to E. A moderate increased size, however, was observed in groups B and C (Table II).

Table II.- Analysis of variance for bursa and thymus body weight ratio (BBWR, TBWR) and antigen/antibody (AG, AB) titer of birds inoculated with different passages and commercial vaccine.

Effect	BBWR	TBWR	AG (10^3)	AB (10^3)
Days (D)	0.092 **	9.40 **	205.60 **	647.19 **
Groups (E)	0.808 **	3.89 **	44.20 **	61.32 **
E×D	0.039 **	0.409 **	5.65 **	14.86 **
SE[1]	0.006	0.014	2.33	21.06

[1]SE = standard error, Significance ** $P < 0.01$.

The results showed a significant difference *(P*<0.0l) among various groups at various time intervals with a significance *(P* < 0.05) interaction of both factors. The data showed that group E differs significantly from rest of groups with maximum thymus body weight ratio. The groups A and D were with moderate with lowest thymus body weight ratio. The thymus body weight ratio was found maximum at 6, 8 and 10th days of post vaccination in A, D and E groups. Groups B and C showed a reduction in size as compared to control group (Table II).

Antigen titre in the bursa

Antigen titre was determined in the bursa of the birds inoculated with different passages of virus, field isolate and commercial vaccine. The virus titre started to increase in all groups (A to E) at 24 h PI relative to the control group and the titre of antigen in group F was at the bottom. The maximum titer of antigen in all groups was obtained at 8th day PI. The titre of virus was high in B and C groups, whereas groups A and E had moderate titre than other groups. Data presented a significant difference (*P < 0.01*) among various groups and various time intervals and also a significant (*P < 0.01*) interaction of both the factors.

Histopathological changes

Bursal changes observed in the groups A to D were minimal, whereas in group E no changes were present at 24 h PI compared with control group F. The significant changes started in groups A to D at 48th h PI to the 8th day. The group E inoculated with 24th passage of virus showed minimal changes at 4th and 6th days PI with respect to the control group F. These changes were necrosis, congestion and hyperplasia of epithelial reticular cells. Lumen contained exudates having degraded cells or cell debris. Affected parenchymal cells were nearly similar in all effected groups (A to D). Accumulation of degenerative mass in medulla and intrafollicular connective tissue proliferation were also observed. Hyperplasia of goblet cells was observed in the pelical epithelial layer, cystic follicles with mononuclear cells infiltration present in the interfollicular area. At the 10th day PI the intensity of changes gradually decreased from sever to mild in the infected groups.

In muscles, changes started at 24 h PI in groups A to D, whereas group E showed no changes compared with control group F. These changes were congestion, hemorrhages, hylanization, dispersed muscles fibres and blood cell in between muscles fibres. The intensity of changes was high in the groups A to D. These changes were observed up to the 8th day PI. At 10th day PI the severity of changes decreased gradually in groups A to D relative to group F.

In the kidneys, no changes were observed in all the groups compared with control group at 24 h PI. The changes started at 48 h PI up to the 10th day PI in groups A to C. In group D changes started at

4th to 10th day PI. These changes include odema, tubular necrosis, intratubular crystalline material (presumable urates) lymphoidfocci and abundant picnotic nuclei in kidneys. While group E showed no changes in the kidneys compared with control group.

In thymus, no changes were observed in all groups up to 24 h post inoculation. At 48 h post inoculation very mild changes were observed in the thymus in groups A to C while, no changes were observed in groups D and E up to 72 h PI. These changes include congestion, slight hemorrhages, necrosis and thickening of intra-lobular connective tissue. Cortical and medullary regions of the thymus were also affected. The changes started at 72 h PI in groups A to D up to the 10th day PI while group E showed no change relative to the control group.

Antibody titre in the birds

The birds were inoculated with three different passages (8, 16 and 24th) of field isolate virus and commercial vaccines to determine the antibody titre. Serum samples from infected birds were collected at 1st, 4th, 6th, 8th, 10th and 14th days PI to check the antibody titre of the birds by indirect haemagglutination (IHA). Data revealed that group E differed significantly from rest of the groups having highest antibody titre, whereas the group F remained at the bottom. The antibody titre was maximum on 14th day PI (Table III).

Challenge protection trial

The birds of all six groups were inoculated with a commercial vaccine, Field isolate and five different selective passages at the age of 21 days. After 15 days of PI, 5 birds of 6 groups (A to F) were challenge with virulent field IBDV having EID_{50} 10 6.3 @ 0.5 ml SIc. The birds were kept 7 days and recorded the protection against challenge (Table IV).

DISCUSSION

The present study was conducted to prepare a safe and effectual vaccine against the drastic effects of IBDV.

Five different passages (8, 12, 16, 20 and 24th), commercial vaccine and field isolates were compared to check the pathogenesis and gross pathological changes on bursa in different groups (A to F) at day one to 10 PI of IBDV. It is clear from the results that pathogenicity of original IBDV reduced considerably after serial passages in embryonated chicken eggs. The complete attenuation of virus and normal body weight ratio of bursa (0.00 mean lesion score) at 24th passage corresponds to the previous observation made by Bayyari *et al.* (1996). The deterioration in size of bursa of all groups was observed and intensity of changes decreased, similarly as reported by Tsukamoto *et al.* (1995) and Bayyari *et al.* (1996). Moody *et al.* (2000) and Tsukamoto *et al.* (1995), reported the pathogenesis of three pathotype vaccines (DV-A, D78, and bursavac) of IBDV, they detected viral nucleic acid in bursa in D78 and bursavac of infected birds at 24, 48, 72 and 120 h PI, however thymus was also positive in D 78 infected birds at 48 h and the bursavac at 72 h PI. The occurrence of D78 in the tissue may be due to the reasons that either maternal antibody might have interfered with the live vaccine or the immunity induced by the live vaccines might simply be insufficient. It indicates that these available vaccines did not induce full protection in the presence of maternally derived antibodies against variant strains. A complete bursal damage in the presence of MDA was reported by Mundt *et al.* (1995), while in the present study only a slight regression was observed due to D78 vaccine. These findings are contradictory due to the IBDV strain, different attenuation level of virus and the status of infection. The local virus belonged to very virulent IBDV pathotype and became attenuated and non-pathogenic to SPF chickens after 24 serial passages. Slight hemorrhages in groups A to D at 24 to 48 h PI and moderate to severe changes was observed in the same groups later at third, fourth and sixth days of PI in thigh and breast muscles. The severity of changes, however, declined from 8th to 10th day PI compared with control group and intensity of changes was slightly different in experimentally induced (Ag) virus. The difference might be due to the strain and the pathogenicity of the virus, as the local strain of the country was trialed. Similarly, Dash *et al.* (1991) reported that gross changes in the bursa were turbid, swollen and enlarged about twice

Table III.- Antibody titre levels at different days in birds inoculated with different vaccines.

Groups	Post inoculation days					
	1	4	6	8	10	14
A (Commercial)	6.4	26.6	53.3	96.0	192.0	426.5
B (Field isolate)	7.2	26.6	53.3	96.0	192.0	426.5
C (8^{th} passage)	6.4	34.6	58.6	106.6	213.0	512.0
D (16^{th} passage)	7.2	37.3	64.0	149.3	213.3	533.3
E (24^{th} passage)	6.4	42.6	74.6	149.3	298.7	597.0

Table IV.- Challenge protection trials of birds inoculated with different selective passage of virus, commercial vaccine and filed isolate.

Groups / Days	1	2	3	4	5	6	7	Mortality	Protection
A (Commercial)	-	-	-	-	-	-	-	0%	100%
B (Field isolate)	-	-	-	1	1	-	-	40%	60%
C (8^{th} passage)	-	-	-	1	-	1	-	40%	60%
D (16^{th} passage)	-	-	-	-	1	-	-	20%	80%
E (24^{th} passage)	-	-	-	-	-	-	-	0%	100%
A (Commercial)	-	-	-	1	2	3	-	100%	0%

than control during 2 to 4 days PI, followed by gradual regression from 5^{th} day. The results reported by Fadly *et al.* (1980), Cheville (1967) and Tanimura *et al.* (1995) are also in line with our findings.

Bursa body weight ratio gradually increased from 1^{st} to 10^{th} day and reaches to the maximum bursa body weight ratio at 6^{th} day PI ranging from 0.72 to 1.23 in the first five groups, whereas in other two groups bursal reduction was recorded at 6^{th} day of PI of IBDV. Similarly Dash *et al.* (1991) reported maximum bursa body weight ratio at 3^{rd} and 4^{th} day PI which was reduced gradually from 5^{th} to 10^{th} day PI. The drop in the mean bursa body weight ratio was a reliable indicator of chronic bursal damage in the later stages of infection. Similarly, Armstrong *et al.* (1981) recorded a drop in mean bursa body weight ratio in commercial flock affected with subclinical IBD. Reduction in bursal weight from fifth day onward, PI in experimental studies was also recorded by Santivatr *et al.* (1981).

No significant changes were observed in thymus and kidney at 24 h PI, however minimal changes in thymus and minimal to mild changes in the kidney were recorded at 48 to 72 h in groups A to D. An increase in the intensity of hemorrhages and reduction in size of thymus, along with enlarged kidneys having urates deposition and bulging of kidney mass was noted at 4^{th} to 8^{th} days PI in the first five treated groups. Both thymus and kidneys reverted to primary size at 10^{th} day PI. These findings are in a fair agreement with those reported by Faragher *et al.* (1972), Henry *et al.* (1980), Ley *et al.* (1983) and Hussain *et al.* (2001). In addition, Cosgrove (1962) observed gross changes in kidneys including swelling and accumulation of urates resulting in their distension. The birds in first five groups (A to E) inoculated with IBDV showed mean thymus to body weight ratio at 2.68 to 1.70 at 6^{th} days PI. While, the other two groups (F and G) showed the same thymus to body weight ratio as that of un-inoculated control group. The thinning of thymus cortex, lymphoid depletion, hemorrhage and reticular cell hyperplasia in the medulla corresponds to the results reported by Rauf (2011). Similar results were obtained by Inoue *et al.* (1994), whereas they differed in the two strain of IBDV. The absence of congestion, severe hemorrhages, exudates in bursal lumen, degraded epithelial cells, and inflammatory fluids in the muscular layer in group E, inoculated with the 24^{th} passage of virus, relative to the control group has already been

reported by Hassan and Saif (1995). Similarly, Nunoya *et al.* (1992) and Starciuc *et al.* (2011) reported severe follicular lymphocytic necrosis along with intense aggregation of neutrophils and hyperplasia of epithelial reticular cells in the bursa. The bursa lumen contained exudates of degenerative pellicle, epithelial cells and neutrophils and pellicle inflammation often extended to the muscular layer and serosa, apparently corresponding to grossly observe gelationous odema of these portions. The lymphocytes were more pronounced in medulla of bursal follicles where 80 to 90% depletion could be seen by 2 days PI as compared to cortex. This was accompanied with introfolicular endothelial cell proliferation and infiltration of heterophils. Accumulation of degenerative mass in medulla and intrafollicular CT proliferation were also observed. The follicles were depleted by the lymphocytes at 4^{th} and 7^{th} day PI, however, no pathological changes was recorded in group E. Hyalinization fibre with loss of strain was observed in heart and breast muscles. Analogous results were observed by some other studies as well (Barnes *et al.*, 1982; Nunoya *et al.*, 1992; Goud *et al.*, 2009). The results reported by Tanimura *et al.* (1995), however, were slightly different with altered changes in the muscles at 5^{th} day of PI to onward. The difference in reduction of hemorrhages, congestion and hyalinization might be due to difference in the pathogenicity of viral strain. In thymus, degeneration and depletion of lymphocytes from medulla at day 5 PI, however, no appreciable change was recorded except small areas of degeneration in medulla 9 day PI. A similar pattern of antibody titer levels were observed in studies by Skeeles and Lukert (1980), Adene *et al.* (1989), and Ahmad *et al*. (2005). The antibody titer at 8, 10 and 14^{th} days of PI resulted in a gradual increase of GMT values in all inoculated groups. At 14^{th} day of PI, a significant higher antibody titer in group E inoculated with 24^{th} passage of virus compared with all other inoculated groups (A to D) is in a fair agreement with those reported by Winterfield and Thacker (1978). Naqi *et al*. (1980) also reported enhanced protection in LKT and BV-M vaccinated birds relative to those vaccinated with BV strains while evaluating the three commercially available vaccines (BV, BV-M and LKT) in broilers. Dash *et al.* (1991) detected antibody from the tissues at 6^{th} to 10^{th} PI and found similar results. The results of the challenge studies showed that groups A and E gave 100% protection followed by group D (80%), B and group C, which gave 60% protection inoculated with 8^{th} passage and field virus.

In conclusion, the absence of histopathological lesion in muscles, thymus, kidney, greater antibody titer level and 100% protection against IBD in group E (24^{th} passage), with respect to the other groups indicates its efficacy to protect the birds from IBDV. On the basis of our results, 24^{th} passage virus can be used to protect the broilers from IBD as cost effective and most economical vaccine.

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(Received 27 December 2013, revised 7 March 2014)