ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 2 (6): 449-453, 2003

Comparative Immune Response Pattern of Commercial Infectious Bursal Disease Vaccines Against Field Isolates in Pakistan

Zaheer Ahmed[1]*, S. Inayat[2], K. Naeem[1] and S.A. Malik[2]
[1]Virology and Molecular Biology Laboratory, Animal Sciences Institute,
National Agricultural Research Centre, Park Road, Islamabad, Pakistan
[2]Quaid-I-Azam University, Islamabad, Pakistan
E-mail: zadani2000@yahoo.com

Abstract: A comparative study was undertaken to evaluate immune response against four types of infectious bursal disease (IBD) vaccines in broilers. The decline pattern of maternal antibody titres in unvaccinated chicks was compared with those given IBD vaccines, following different vaccination schedules. Indirect ELISA as well as challenge with field virus evaluated the immune response of these vaccines. The results indicated that immune response against different vaccines varied in accordance with the vaccine schedule and levels of maternal antibody against IBDV in the chicks. The challenge studies indicated that only those birds stayed refractory to the challenge in which either sufficient IBDV maternal antibody titres were present or the birds were challenged at least 2 weeks post IBDV vaccination, providing sufficient time for developing an effective immune response. So it is suggested to devise IBD vaccination schedule in the light of prevailing maternal antibody tires in day-old chicks of each flock.

Key words: Gumboro disease, IBD ELISA, IBD-vaccine

Introduction

Infectious bursal disease (IBD) has become a major poultry disease in Pakistan during the past 5-7 years, primarily in layers and broilers. The disease has shown mortality, sometimes as high as 23% in a few isolated outbreaks (Anjum *et al.*, 1994). The disease itself causes a variable degree of immunosuppression in the affected birds. When the chickens are infected at an early age, they display a severe and prolonged immunosuppression, compromising both humoral and cellular responses of chickens (Lukert and Saif, 1991). Infectious bursal disease virus (IBDV) also has a potential for antigenic heterogeneity, which results in frequent outbreaks in the field, even in the flocks vaccinated against IBD (Hasan *et al.*, 1998). The IBDVs isolated from these outbreaks have shown to differ antigenically and pathogenically from the standard IBDV. They have been designated as variants of the original strain. Some of these isolates have shown highly pathogenic form of IBDV i.e. very virulent IBDV (vvIBDV) in Europe, Middle East, Asia and Africa (Shane, 1993).

One of the significant components of the control of IBD is its vaccination, which if improved may help in lowering the incidence of IBD in poultry. All types of IBD vaccines used in Pakistan are imported and comprise of a variety of vaccine strains. However, a variety of vaccination schedules have been devised for their use. Despite the regular use of these IBD vaccines, the disease still prevails in the country and affects the flocks of different age groups.

In the present study, using different vaccination schedules along with considering maternal antibody titres against IBDV, attempt has been made to evaluate the potency of three imported IBD live-vaccines when used alone or in combination with a killed aqueous IBD-vaccine developed from locally isolated IBDV.

Materials and Methods

Birds and Housing: Commercial broiler chicks obtained from M/S Sadiq Brothers (SB), Chicks (Rawalpindi), of Hubbards strain were used in this study. The chicks were reared in isolated rooms at the Animal House facility of Animal Sciences Institute, National Agricultural Research Centre (NARC), Islamabad. The chicks were given feed and water *ad libitum*.

Preparation of Virus Inoculum: Bursa and spleen were collected from IBDV affected chicks from the field and were stored at -20 °C until used. A 20% (w/v) bursa and spleen homogenate was prepared by blending them in sterile phosphate buffered saline (PBS pH=7.2) along with antibiotics (Penicillin 1000 IU/ml, Streptomycin 100 µg/ml Gentamycin 10 µg/ml and Fungizone 2.5 µg/ml). The preparation was freeze-thawed thrice and centrifuged at 2000 rpm for 10 minutes. The supernatant was collected and filtered via 0.22 µm syringe filter. The virus was tested against known positive antisera of IBDV by Agar Gel Precipitation Test (AGPT). The positive

samples were stored at -20 °C.

The viral preparation was then inoculated onto chicken embryo fibroblast (CEF) cells that were prepared following the standard procedures (Rovozzo and Burke, 1973). After 4-5 days of incubation, the cells were freeze-thawed thrice followed by re-inoculation onto CEF and examined for 4-5 days, or till the production of CPE. The flasks were freeze-thawed thrice and the lyzed cells were centrifuged. The supernatant was collected as stock virus titrated in CEF. For the challenge experiment IBDV titre of $10^{5.5}$ $TCID_{50}$ was used (Reed and Muench, 1938). The supernatants containing IBDV were then stored at -70 °C.

Preparation of Vaccines: Three commercially available live vaccines namely, Gumboro Vaccine Nobilis strain D-78 prepared from (Intervet) [(A)], IBD-Vac (Choong and Animal Disease Lab., Korea) [(B)] and Gumboro vaccine Nobilis strain 228-E from (Intervet) [(D)], were used in this study. These were live vaccines and used according to the manufacturer's directions. A killed vaccine (C) comprising of a field isolate of IBDV virus (NARC-ASO5) was used for comparison purposes. The virus was used in a titre inducing ≥ 12.5 log_2 ELISA units/dose. This vaccine was injected subcutaneously. Blood was collected from all the birds, including uninoculated control birds, at weekly interval starting from day one of age.

Preparation of Antisera: Four, 20-day old chicks kept in isolation were vaccinated via eyedrop route with $10^{6.4}$ EID_{50} of IBD live vaccine using Nobilis strain 228-E of M/S Intervet. Fifteen days post-vaccination, the above vaccine was inactivated using formaline and emulsified with Fruend's complete adjuvant as per standard protocol. Out of it, 4 birds of 15-day age were re-vaccinated subcutaneously. After another fifteen days the birds were bled and tested by AGPT for the presence of antibodies against IBDV as well as to decide if revaccination was needed before the final sera collection.

Serological Assessment: Agar gel precipitation test (AGPT) was performed against all sera samples following the standard protocol of Crowle (1973). Enzyme linked immunosorbent assay (ELISA) was performed by first determining the working dilutions of IBDV antigen, serum and conjugate, using flat bottom polystyrene micro titration plates. The ELISA plates were coated with IBDV antigen, prepared by the method described by Voller *et al.* (1989). For this purpose antigen was coated onto each well using 100ul/well of antigen solution. Uncoated sites were blocked with 0.1 M NH_4Cl solution by incubating at 37 °C for one hour. The plates were washed with PBS-Tween 20. The test was serially diluted in PBS for titration. Plates were incubated at 37 °C for one hour followed by washing with PBS-Tween-20 twice. To each plate a variable dilution (i.e. 1:500 to 1:1000, 1:2000, 1:3000, 1:4000) of Rabbit, anti-chicken IgG conjugated with HRP was added in appropriate wells. The plates were then incubated at 37 °C for one hour and eventually washed twice with PBS-Tween-20. Orthophenylene diamine dihydrochloride (OPD) dissolved in phosphate citrate buffer was used as substrate which was added to the wells and plates kept in dark for 15 minutes. The reaction was stopped using 2M H_2SO_4.

The plates were read on ELISA reader at 492 nm and absorbance of each sample was recorded. The highest dilution of antigen with the highest dilution of positive serum giving maximum absorbance was identified to be the ideal concentration of IBDV antigen for coating the ELISA plates for further evaluation of unknown serum samples. Later on ELISA was done on experimental samples using the above procedure.

Data analysis: The sample to positive (s/p) ratio of the samples, i.e. the absorbance value of the test serum divided by the absorbance value of the positive control serum was calculated to interpret the results.

Experimental Design: The trial was divided into three experiments.

Experiment 1: The objective of this experiment was to monitor the declining pattern of maternally derived antibody (MDA) against IBDV in chicks. For this purpose, fifty broiler chicks of day-1 were reared in isolation. Ten chicks randomly selected from this group at each occasion and bled (after every week) up to 42 days of age. After the blood coagulation the serum was separated and saved for serological testing. Furthermore, randomly selected five chicks from this group were separated every week and challenged with the purified IBDV up to 6 weeks of age. Birds that died during the experiment were necropsied otherwise they were bled one week after challenge and the antibody titre against IBDV was determined.

Experiment 2: In this experiment the effect of IBD vaccines on the depletion rate of IBDV maternally derived antibody (MDA) titres in the chicks, along with the immune response in the IBDV vaccinated birds was examined. The above-mentioned four IBDV-vaccines (A - D) were used during this experiment. This experiment was conducted in three parts. In part 1, a total of 120

Table 1: Declining pattern of maternal derived antibody titres (MDA) at different ages of chickens

	Mean±SD of serum antibody titres of MDA at different time interval					
	Week-1	Week-2	Week-3	Week-4	Week-5	Week-6
ELISA s/p ratio*	0.484±0.088	0.468±0.089	0.280±0.176	0.202±0.184	0.182±0.033	0.155±0.042

*s/p = sample/absorbance of positive. N = 10 birds examined in each week.

Table 2: Immune response in unvaccinated group upon challenge with the field IBDV

	Challenge age, mortality and immune response weeks post challenge (PC)					
	Week-1 n=5	Week-2 n=5	Week-3 n=5	Week-4 n=5	Week-5 n=5	Week-6 n=5
ELISA s/p ratio	0.377±0.222	0.552±0.121	0.453±0.084	0.661±0.055	0.799±0.253	0.832±0.113
Mortality due to IBDV	0/5	0/5	0/5	0/5	0/5	0/5

birds were divided into two groups of 60 each. Out of this, each sub-group comprising of 12 birds each were vaccinated separately on day 1 with either of the vaccines labeled as A, B, C and D. The remaining 12 birds acted as control. The other 60 birds were vaccinated in the same manner as detailed above but on day 10 of age. Twelve birds were also kept as non-vaccinated control. From each group, 5 birds were bled every week starting from 2 weeks of age.

In part 2, the birds were divided in subgroups similar to part 1 and vaccinated at days 1 and 21 with the four vaccines, one vaccine to 12 birds in each group. For part 3 of the experiment, another group of 60 chicks was reared separately and grouped as detailed above for vaccination with four vaccines but vaccinated at day 10 and day 21 of age. After weekly bleedings, the sera were separated and stored at -20 °C till used.

Experiment 3: In this experiment the response of birds from experiments 1 and 2 upon challenge with live field isolate of IBDV was examined. For this purpose birds (3-5) from each group were weekly shifted to a separate isolation room and given injection of IBDV field isolate ($10^{5.5}TCID_{50}$/bird) accordingly. The birds were examined for any clinical signs or death and they were subsequently bled at one week after the challenge. The sera were collected and tested for antibody levels against IBDV.

Results and Discussion

It was important to determine the working dilutions of the IBDV antigen used for coating the multi-well plate for ELISA. It was determined to be 0.1 ml of the stock. The concentration of positive serum and conjugate was determined to be 1:80 and 1:4000, respectively. The purpose of this study was to examine the maternal antibody levels of anti-IBDV antibodies in the progeny chicks which would then serve as the base line when the chicks were challenged with the commercial or local vaccines. In first series of experiments, progeny chicks (unvaccinated) were tested at 1, 2, 3, 4, 5, and 6 weeks of age. This was done to assess the natural rate of maternal-derived antibody decay in these chicks. As can be seen from the data presented in Table 1, the maternal anti-IBDV antibody levels were fairly high at 1 and 2 weeks post hatch. These levels were almost two-fold greater than the ones observed at 3 weeks of age or onwards. The antibody levels declined rapidly after two weeks of age, however, were still detectable by the end of the study (i.e., 6 weeks). This is not surprising since these chicks were not raised on specific pathogen free environment and were perhaps still exposed to low levels of antigenic challenge from external sources. This level of decline in passively transferred IBDV antibodies is not surprising. Similar levels of maternal antibody decay were observed in one of our previous studies (Ahmed and Akhter, 2003). The reasons for such declines may be several fold, the proteolytic degradation of antibodies or neutralization due to naturally occurring/persisting IBDV perhaps would be the foremost.

In the second series of experiments, the progeny chicks (unvaccinated) were challenged with a field isolate of IBDV at every week up to six weeks of age. The data are presented in Table 2. As can be seen, the antibody levels were much lower in chicks of one week of age. In chicks of ages 2 to 6 weeks, the antibody levels upon challenge increased significantly, suggesting that the maternal antibody levels had minimal or no effect on seroconversion against the field isolate used as the vaccine antigen. These observations also suggest that if there was any protective effect of the maternally-derived anti-IBDV antibodies, it would diminish after one week of age. Kenzevic *et al.* (1987) showed that MDA persisted

Table 3: Serological response of birds against IBDV vaccines given at variable time intervals

Vaccine Type	Vaccination Schedule (Day)	Post Vaccination ELISA Immune Response At Different Age			
		WEEK 3	WEEK 4	WEEK 5	WEEK 6
	1	0.428 ± 0.101	0.211±0.112	0.121±0.120	0.088±0.083
A	10	0.277±0.331	0.527±0.198	0.973±0.290	0.992±0.231
	1+21	0.484±0.641	0.800±0.172	0.667±0.158	0.805±0.188
	10+21	0.488±0.078	0.456±0.257	0.9360.201	1.099±0.127
	1	0.421±0.121	0.282±0.171	0±.181±0.171	0.191±0.119
B	10	0.225±0.223	0.726±0.056	0.856±0.142	1.013±0.131
	1+21	0.394±0.111	0.507±0.186	0.964±0.112	0.935±0.222
	10+21	0	0.437±0.200	0.737±0.037	1.141±0.079
	1	0.286±0.132	0.243±0.082	0.211±0.195	0.135±0.087
C	10	0.514±0.391	0.721±0.289	1.019±0.143	0.847±0.140
	1+21	0.411±0.034	0.432±0.148	0.754±0.239	0.935±0.176
	10+21	0	0.347±0.111	0.884±0.035	1.070±0.281
	1	0.262±0.115	0.202±0.018	0.188±0.122	0.150±0.153
D	10	0.117±0.050	0.407±0.195	1.024±0.223	1.074±0.050
	1+21	0.392±0.121	0.401±0.292	0.735±0.296	1.025±0.176
	10+21	0	0.391±0.286	0.801±0.173	1.051±0.108

Note: no sampling was done during week 1 & 2 of age in single shot group and in 3 week of age in group with double shot.

A = IBD Nobilis strain D-78 (Intervet, Netherland) B = IBD-Vac (Choong and Animal Disease Lab, Korea)
C = IBD-NARC strain (local) D = IBD Nobilis strain 228-E (Intervet, Netherland)

upto 6th weeks of age and Rosales *et al.* (1989) thereafter reported IBDV infection at 15th day of age in the presence of MDA. It means that similar situation prevails under our local condition in Pakistan where birds are liable to get infection at an early age due to high field challenge.

The data in Table 3 represents the post-vaccine ELISA titres determined at 3 - 6 weeks post vaccination. The birds were vaccinated using a vaccine regimen of vaccinating at day of hatch, at 10 days of age, vaccinating at day 1 and day 21 of age and vaccinating at day 10 and day 21 of age. The results show that vaccines A, B and D representing live IBDV, did not produce an immune response when given at day 1 as the pattern of antibody level in these vaccinated groups was similar to the unvaccinated control (Table 1). There was no apparent increase in the IBDV antibody levels in group C given killed vaccine as well.

In the group vaccinated at 10th day of age, the three vaccines A, B and D produced low titres up to 3rd week, however, the titres increased from 4th week onward (Table 3). The antibody titre ranged between 0.527 to 1.0992 as compared to 0.088 to 0.282 among groups given a single injection at day 10. In case of the killed vaccine (C), better immune response was produced from 3rd week onward. Table 3 also shows the immune response against IBDV vaccine given as two vaccine injections. The group given vaccines on day 1 and 21 of age produced antibody titres ranging between 0.432 to 1.025 against all types of vaccine. The overall ELISA antibody titres developed against all the four IBDV vaccines by 6 weeks of age indicated that a single injection of vaccine at day 1 produced the lowest immune response (0.088±0.083) whereas, the highest antibody titres were shown by using two injections of vaccines at day 10 and 21 days of age.

While the seroconversion response of the birds against various vaccines was variable over time, no mortality was observed in any of the vaccinated group upon challenge with the live field isolate of IBDV (data not shown). Similar observations have been reported earlier by Naqi *et al.* (1982). Our findings indicate that none of the vaccines used were capable of inducing satisfactory serologic response, nor they was able to confer protection (in terms of inducing neutralizing antibodies as detected by ELISA) when used at day 1 of age. On the other hand, the birds which were not vaccinated at all became exhibited signs of infection after 3rd week of age. The titres developed by the birds after vaccinating with the killed vaccine persisted throughout the experimental period of six weeks and also withstood the challenge. The weak protection induced by vaccine D eventually resulted in a very high anamnestic response at 3rd week of age after using challenge dose of field virus. As this vaccine (D) contained a more invasive strain of IBDV (228-E), it should have penetrated the higher MDA titres

to establish better immune response upon vaccination at day 10. However, it eventually produced a better immune response after 4th week of age. While comparing the results of single-shot with two-shot vaccination, it was found that two-shot procedure induced better and longer lasting immune response. Here the immunized chickens responded more effectively against the challenge virus in all the age groups upto 6 weeks, thereby showing the ability of this protocol to resist the field challenge more effectively.

References

Ahmed, Z., and S. Akhter, 2003. Role of maternal antibodies in protection against infectious bursal disease in commercial broilers. Int. J. Poult. Sci., 2: 251-255.

Anjum, A.D., G.S. Sabri and K. Jamshidi, 1994. Occurrence spread and control of infectious bursal disease in Pakistan. In Proceedings of 1st PPA-PVMA Punjab, International Poultry Conference March 30 - April 01, pp: 57-59.

Crowle, A.J., 1973. Immunodiffusion. Academic Press, New York.

Hasan, S., K. Ahmad, N. Fawad and B. Siddique, 1998. A study on the infectious bursal disease around Rawalpindi/Islamabad. Pak. J. Live. Res., 8: 84-88.

Kenzevic, N., D. Rogan, Matovic and B. Kozlina, 1987. Persistence of maternal antibodies in the yolk and serum of chick from parents vaccinated and infected with infectious bursal disease virus. Vet. Glasnik., 41: 767-773.

Lukert, P.D. and Y.M. Saif, 1991. Infectious bursal disease. In: Diseases of poultry 9th ed. Iowa State University Press, Iowa, USA, pp: 648-663.

Naqi, S.A., B. Marquez and N. Sahin, 1982. Maternal antibody and its effects on infectious bursal diseases immunization. Avian Dis., 27: 623-631.

Reed, L.J. and H. Muench, 1938. A simple method of estimating fifty percent end points. Am. J. Hyg., 27: 493-497.

Rosales, A.G., P. Villegas, P.D. Lukert, O.J. Fletcher, M.A. Mohammed and J. Brown, 1989. Pathogenicity of recent isolates of infectious bursal disease virus in specific pathogen free chickens: Protection conferred by an intermediate vaccine strain. Avian Dis., 33: 729-734.

Rovozzo, G.C. and C.N. Burke, 1973. Tissue culture propagation. In: A manual of basic virology techniques. 1st ed., Prentice-Hall, Inc., Englewood Cliffs, N.J., pp: 48-55.

Shane, S.M., 1993. Prevention and control of infectious bursal disease in Asian countries. Technical Bulletin of American Soybean Association, 518: 2-7.

Voller, A., D.E. Bidwell and A. Barttett, 1989. The enzyme linked immunosorbent assay (ELISA). Nuffield Laboratories of comparative Medicine. The Zoological Society of London, Reagent's Park, London, NW1.