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 3 (4): 300-304, 2004

Interaction Between Newcastle Disease and Infectious Bursal Disease Vaccines Commonly Used in Sudan

A.S. Ali, M.O. Abdalla and M.E.H. Mohammed
Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Medicine, University of Khartoum, Khartoum North, P.O. Box 32, Sudan
E-mail: abumalaz2002@yahoo.com

Abstract: It was the aim of this study to determine the interaction between the Newcastle disease (ND) and infectious bursal disease (IBD) vaccines used to control these two important viral infections greatly affecting poultry industry worldwide. The commercially available vaccines in the Sudan were used. Haemagglutination inhibition (HI) and agar gel immunodiffusion (AGID) tests were employed to measure the Ab titres in chicks sera to ND and IBD respectively. Generally, IBD vaccine was reported to have adverse effect on the ND vaccine whereas the reverse was not true. The results obtained also revealed that better Ab responses against NDV were detected when ND vaccine was administered before IBD vaccine. The deleterious effect of IBD vaccine on Ab levels against NDV antigens was slightly ($p< 0.05$) low when IBD vaccine is administered at two weeks as compared to three weeks of chicken age. No variations in the Ab titres when chicks were boostered with ND vaccine containing LaSota or Komorov strain of the virus at 4 weeks were observed. However, slightly ($p< 0.01$) better Ab responses were noted for LaSota over Komorov strain. It was, therefore, concluded that vaccination of chicks with ND vaccine containing LaSota strain of the virus when they were 10 days followed by vaccination with IBD vaccine at two weeks and boostering with the same ND vaccine yielded better Ab responses but slightly lower protection levels.

Key words: Newcastle disease, infectious bursal disease, antibody, vaccines

Introduction

Newcastle disease (ND) is a contagious, highly fatal viral infection affecting many species of domestic and wild birds worldwide and regarded as an Office Internationale des Epizooties (OIE) list A disease (Office Internationale des Epizooties, 2001). This is due to the huge economic impact on poultry industry precipitated following outbreaks of the disease (Aldous and Alexander, 2001). Infectious bursal disease (IBD) also known Gumboro disease is an acute highly contagious viral infection, particularly important for young chicks (Parkhurst, 1964; Lukert and Saif, 1991). Outbreaks of IBD with high mortality due to very virulent strains of the virus were reported in Europe (Chettle *et al*., 1989) and other parts of the world (Hair-Bejo, 1992; Nakamura *et al*., 1994). The significant concern of the disease did arise to high fatalities, reduced productive and reproductive efficiencies among infected chicks (Shane *et al*.,1994) as well as the immunosuppressive activity of the causative virus resulted from severe damage of bursa of Fabricius (Faragher *et al*., 1974; Sharma *et al*., 2000). Proper vaccination programmes and strict biosecurity measures were proved in many reports as essential tools for the control of these two diseases (Giambrone and Clay, 1986; Wyeth and Chettle, 1990; Whitfill *et al*., 1995; Haddad *et al*., 1997).

The immunosuppressive effects of IBDV had previously been reported to adversely affect vaccination against ND (Allan *et al*.,1972; Faragher *et al*., 1974; Giambrone *et al*., 1976) and other viral infections (Li-Wei Jen and Cho, 1980; Yuasa *et al*., 1980). The objective of the present study is to determine the interaction between ND and IBD vaccines commonly applied in Sudan to control these diseases. Particular focus on the effect of live IBD vaccine on the efficacy of ND ones using different vaccination schedules was made.

Materials and Methods

Chicks: Two hungered and fifty, one-day old, White Hissex broiler chicks were obtained from the Arab Company for Agricultural Production and Processing (Khartoum, Sudan). They were reared and raised in slatted cage in experimental house for the purpose of this study.

Vaccines: The vaccines used in the study were as follow:

Live NDV vaccine containing the lentogenic chick-embryo propagated, LaSota strain of the virus.

Live NDV vaccine containing the merogenic chick-embryo propagated, Komorov strain of the virus.

Live IBDV vaccine containing the chick-embryo propagated, D78 intermediate strain of the virus.

The first and third vaccines were purchased from Intervet Holland (Intervet International Boxmeeer-Holland) whereas the second one was locally produced and kindly supplied by the department of viral vaccines production at the Central Veterinary Research Laboratory

(CVRL) (Khartoum, Sudan). All the vaccines were received in a freeze dried state and dehydrated just prior to use as recommended by the manufacturer.

Virus: The previously known virulent Herts 33/56 strain of NDV (Alexander, 1991) was used in this study to challenge the vaccinated chicks. The virus was obtained from the virology department, Central Veterinary Research Laboratory (CVRL) (Khartoum, Sudan) in a vial containing freeze-dried allantoic fluid.

Preparation and titration of NDV (Herts 33/56 strain): The content of the vial was reconstituted in 5ml of physiological saline. Each 0.2ml of the reconstituted virus was inoculated in the allantoic cavity of 9-11 day old embryonated chicken eggs. Five days post-inoculation, the amonioallantoic fluids (AAF) of dead and live embryos were collected and examined by HA and HI tests for virus growth using a known NDV antiserum. To determine the EID_{50} of the virus, ten-fold serial dilutions (10^{-1}- 10^{-9}) were prepared from the harvested virus in normal saline. For each dilution, 0.1 ml was inoculated in five, 10 day-old, embryonated chicken eggs. Deaths of embryos occurred 24 hours post-inoculations were recorded and EID_{50} of the virus was calculated according to the method of Spearman-Karber (Finney, 1964).

Vaccination of chicks: Water was withheld from the birds for a number of hours before the vaccine application. The ND (LaSota) and IBD vaccines were given to the birds in a fresh drinking water at a concentration carefully calculated to give each bird a sufficient dose according to the manufacturer's instructions. The ND (Komorov) vaccine was administered via the intranasal route as recommended by the manufacturer.

Blood sampling: The blood was collection from the wing vein of chickens using 5 ml disposable syringes. Collected blood was left for 2-4 hours at room temperature to clot, after which the clot was loosened. It was then kept at 4°C over-night, separated the following day by low speed centrifugation. The sera after separation were stored at –20°C till needed. Blood was sampled from chicks at the age of 10, 14, 21, 25, 28, 42, 53, 63 and 69 days. Blood samples collection at 10, 14, 21, 28 and 53 days of chicken age was performed just before the vaccination and challenge of birds.

Haemagglutination inhibition (HI): The HI test was carried out according to the well-established principles and protocol of Allan *et al.* (1978). The modifications made by Abdalla *et al.* (1999) were also followed in this study. Two-fold serial dilutions of serum samples were made with normal saline in microtitre plates. Volumes of 0.05 ml of a known NDV antigen (PHI DooRn ND HI antigen I ml 3089 Intervet, Holland) containing 4 haemagglutinating units were added in each well of the plate. Three rows of wells were left as controls: the first row contained a known NDV antiserum (positive control), the second row contained NDV antigen without serum (negative control) and the third row contained normal saline with RBCs (reagent control). The plate was shaken and left for 30 minutes at room temperature before 0.05 ml of chicken RBCs to each well was added. The plate was then rotated and left for 20 minutes or till a pattern of HA appeared. HI titres were expressed as the reciprocal of the highest dilution that cause 50% inhibition of agglutination. The base two logarithmic titre was then calculated.

Agar gel immunodiffusion (AGID) test: The test was performed to detect the Ab responses to IBDV in chicks sera essentially as described by Intervet Laboratories, Holland. A known reference IBD antigen (PHI- DooRN IBD AGPT antigen 0291 Intervet Holland) was placed in the inner well of the gel. Sera under test were placed in the outer wells adjacent to a known positive serum (PHI DooRN S 92006 IBD serum, Intervet Holland) as positive control. The gel was then incubated in a humidified chamber at room temperature and examined for precipitation lines 24-48 hour later.

Experimental design: The chicks were divided into five groups (A, B, C, D and E) with 50 chicks per group. Groups, A, B, C and D are the vaccinated groups whereas group E was left without vaccination as control. The vaccination schedule and challenge of birds is demonstrated in Table 1. When chickens were 53 days old, each bird in all groups was inoculated intramuscularly with 1 ml containing $10^{7.5}$ EID_{50} of Herts 33/56 strain of NDV for challenge. Following challenge, birds were observed for 16 days, clinical signs and mortalities were recorded. At 10 and 16 days post-challenge, randomly selected birds from each group were scarified and examined for ND lesions.

Statistics: The data obtained in the study was analyzed using the two-way and one-way analysis of variance (ANOVA) so as to determine the significance of differences between groups of data.

Results

Maternally derived Ab (MDA) against NDV: The geometric mean of MDA against NDV in all groups of chickens, as measured by HI test at 10 day old of chicks, is depicted in Fig. 1. It is observed to range between 5.42±0.12 (for group C) and 5.04±0.18 (for group B). No significant variation ($p< 0.05$) among different groups of chicken in their MDA level.

Titration of Herts 33/56 strain of NDV: The EID_{50} of

Table 1: Vaccination schedules for different groups of chicks

Chicken age (day)	Group of Chickens				
	A	B	C	D	E
10	ND-LaSota	IBD	ND-LaSota	ND-LaSota	
14	IBD	ND-LaSota		IBD	
21			IBD		
28	ND-LaSota	ND-LaSota	ND-LaSota	ND-Komorov	
53	Challenge with Herts 33/56 strain of NDV				

Birds in group E were left through the experiment without vaccination as control

Table 2: Detection of Ab response to IBDV using AGPT before and after vaccination

Chicken age (day)	Group of Chickens				
	A	B	C	D	E
10*	41.67**	91.00	41.67	66.60	58.33
28	50.00	58.00	58.00	50.00	00.00
42	66.60	58.00	58.00	50.00	00.00

* Abs against IBD detected at 10 days old of chicks (before vaccination) are the MDA

** (No. of positive sera/ No. of sera tested) %. A number of 12 sera were tested for each group of chickens; n=12.

Herts 33/56 strain of NDV which used to challenge the vaccinated birds is $10^{7.5}$ EID_{50}/ ml.

Morbidity and mortality: No obvious clinical signs for ND and IBD were observed following administration of the vaccines. Total of five birds (2 from group A, 2 from group C and one from group E) had died after vaccine application (before challenge) but no evidence of ND or IBD lesions in dissected dead birds.

Antibody responses to ND vaccines: The Ab titres as detected by HI in all groups of chicks following vaccination with ND and IBD and challenge are demonstrated in Fig. 1. Antibody titres in chickens group A in increased following primary vaccination with ND-Lasota vaccine but decreased after IBD vaccination. This situation slightly reversed in group B indicating the influence of IBD vaccine on Ab response to NDV. The results obtained in group C indicated the lower effect of IBD vaccine on Ab response to NDV when administered at 21 day compared to 14 day old (as in group A). Comparable results of group A and D of chickens are observed, although slightly better ($p< 0.01$) Ab responses were noted when birds given ND-LaSota over ND-Komorov vaccine as a secondary dose. In the control group (E), the Ab titre was significantly decreased ($p<0.05$) when chicks were 10 days old (5.32±0.21) up to 25 days old (2.88±0.43) and remained low till challenge. Similar trend, for all groups of chickens, described by a decrease of Ab titres to ND following challenge of birds with the virulent strain of NDV was observed except for the control where increase in the titres is obtained.

Antibody responses to IBD vaccine: The Ab responses to IBDV, detected by AGPT, in all groups of chickens are shown in Table 2. The MDA is almost comparable in all groups except for group B where is detected in 91.00%. Generally, no effect of ND vaccines on the presence of Ab responses to IBD can be seen in the data obtained.

Protection: Following challenge of vaccinated birds, protection levels of 87.50, 94.00, 89.58, 96.00 and 63.26 were calculated in group A, B, C, D and E respectively. Classical ND lesions were observed in the scarified and dead birds.

Discussion

Newcastle disease (ND) and infectious bursal disease (IBD) pose great hazard threatening poultry industry in many parts of the world. In Sudan, a lot of vaccines had been introduced to control them with failures encountered from time to time. The salient question addressed in this study was to determine the interaction between the most commonly used vaccines against the diseases and its role in vaccination failure. It was well established that maternally derived antibodies (MDAs) are protective against ND infection (Allan *et al.*, 1978). The chicks used in the present study were laid by hens with a history of vaccination, hence appreciable amounts of Abs are detected in all groups of chickens before vaccination (5.42±0.12-5.04±0.18) with minor variations among the groups tested. This MDA detected is also found to protect chicks against residual effect of the ND-LaSota vaccine when employed during primary vaccination which proved previously to have some pathogenic effects in vaccinated chicks (Murphy *et al.*,1999).

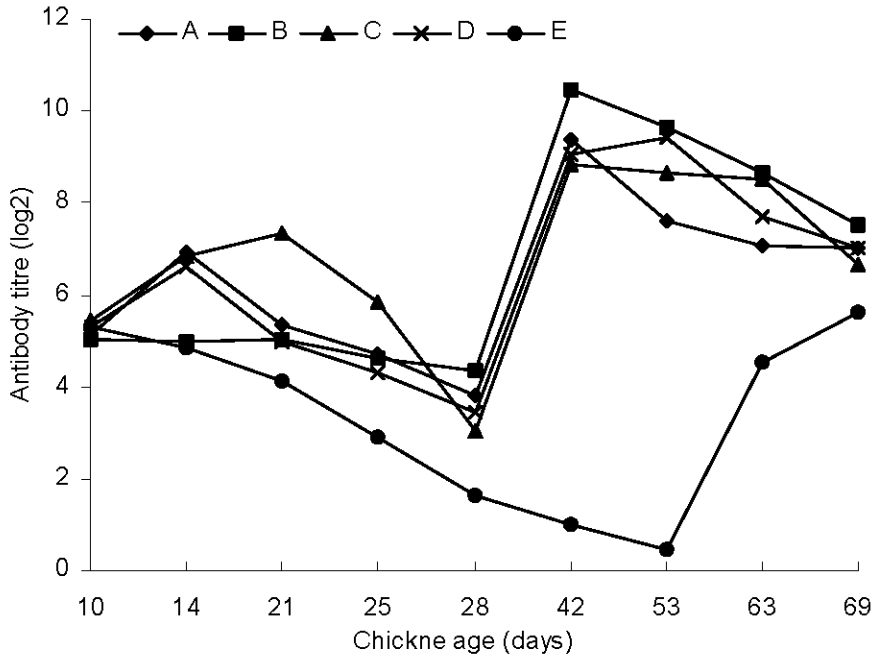

Fig. 1: Antibody titres against NDV as detceted by HI test

The results obtained in this study also showed that higher Ab levels against NDV, detected by HI, are observed when ND vaccine was applied before IBD one (group A & B) but the reverse is true for protection levels obtained following challenge. This suggests the immunodepressive effect of live IBD vaccine on ND vaccine due to the damage in the bursa of Fabricius and substantiate many previous reports (Allan *et al.*,1972; Faragher *et al.*, 1974; Giambrone *et al.*, 1976). It also suggests Abs are not the only factors that mediate protection against ND, however, CMI is also greatly involved.

This immunodepressive effect of IBD vaccine on vaccination against ND is detected less profound when chicks vaccinated with IBD vaccine at two weeks compared to three weeks of chicks age. This is in agreement with Lukert and Mazariegos (1985) who sated that the intermediate strains of IBD vaccine can induce bursal atrophy and immunosuppression in three weeks old of chicks. It is not possible to detect significant variations in the Ab levels when chicks boostered with LaSota or Komorov-ND vaccine although chicks boostered with LaSota stimulated slightly better ($p< 0.01$) Ab responses compared to those boostered with Komorov. This may partly attributed to the antigenic dissimilarities between the two strains of the virus and partly due to the individual variations among chicks to respond to viral antigens which confirmed in the past time (McKeever *et al.*, 1987).

No obvious adverse effect of ND vaccines on Ab stimulation to IBDV antigens, when detected using AGPT, was observed in this study. This might not be possible because the test employed is qualitative rather than to quantify the Ab titres against IBDV in chicks sera. VNT (Ismail and Saif, 1990) and ELISA (Zaheer and Akhter, 2003; Hair-Bejo *et al.*, 2004) are proved more sensitive to measure Ab titres against IBD vaccines, hence recommended for use in future experimentation. It was concluded that vaccination of chicks with ND vaccine containing LaSota strain of the virus adversely affected by live IBD vaccine when administered first. At any rate, the vaccination programme employed in group A of chickens is recommended for use under Sudan conditions since good Ab titres and protection levels for the vaccines were obtained.

References

Abdalla, M.O., M.E.H. Mohammed, A.S. Ali, M.M. Mukhtar and M.L. Mohd-Azmi, 1999. The immunostimulatory effects of Levamisole and egg white powder on humoral and cellular immunity to Newcastle disease vaccination. Malaysian Appl. Bio., 28: 73-77.

Aldous, E.W. and D.J. Alexander, 2001. Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). Avian Path., 30: 117-128.

Alexander, D.J., 1991. Newcastle disease, In: Newcastle disease vaccines for Rural Africa. Edited by M. M. Rweyemamu, V. Palya, T. Win and D. Cylla. pp: 7-45. Proceeding of a workshop held at Pan African Veterinary Vaccine Centre. Debre Zeit, Addis Ababa, Ethiopia 22-26 April 1991.

Allan, W.H., J.T. Farragher and G.A. Cullen, 1972. Immunosuppression by the infectious bursal agent in chickens immunized against Newcastle disease. Vet. Rec., 99: 511-512.

Allan, W.H., J.A. Lancaster and B. Toth, 1978. Newcastle disease vaccines: their production and use. FAO Anim. Prod. Ser. No. 10, FAO, Rome.

Chettle, N., J.C. Stauart and P.J. Wyeth, 1989. Outbreak of virulent infectious bursal disease in East Anglia. Vet. Rec., 125: 271-272.

Faragher, J.T., W.H. Allan and C.J. Wyeth, 1974. Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease. Vet. Rec., 95: 385-388.

Finney, J.D., 1964. Statistical method in biological assay. 2nd ed., Griffin, London, pp: 525-531.

Giambrone, J.J. and R.P. Clay, 1986. Vaccination of day-old broiler chicks against Newcastle disease and infectious bursal disease using commercial live and/or inactivated vaccines. Avian Dis., 30: 557-561.

Giambrone, J.J., Eidson, C.S., Page, R.K., Flecher, O.J., Barger, B.O. and S.H. Kleven, 1976. Effect of infectious bursal agent on the response of chicken to Newcastle disease and Marek's disease vaccination. Avian Dis., 20: 534-544.

Haddad, E.E., C.E. Whitfill, A.P. Avakian, C.A. Ricks, P.D. Andrews, J.A. Thoma and P.S. Wakenell, 1997. Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. Avian Dis., 41: 882-889.

Hair-Bejo, M., 1992. An outbreak of infectious bursal disease in broilers. J. Vet. Malaysia, 4: 168.

Hair-Bejo, M., M.K. Ng and H.Y. Ng, 2004. Day old vaccination against infectious bursal disease in broilers chickens. Int. J. Poult. Sci., 3: 124-128.

Ismail, N.M. and Y.M. Saif, 1990. Differentiation between antibodies to serotype 1 and 2 Infectious bursal disease virus in chickens sera. Avian Dis., 34: 1002-1004.

Li-Wei Jen and B.R. Cho, 1980. Effects of infectious bursal disease on Marek's disease vaccination: Suppression of antiviral immune response. Avain Dis., 24: 890-906.

Lukert, P.D. and L.A. Mazariegos, 1985. Virulence and immunosuppressive potential of intermediate vaccine strains of Infections bursal disease virus. J. Am. Vet. Med. Assoc.,187: 306-312.

Lukert, P.D. and Y.M. Saif, 1991. Infections bursal disease. In disease of poultry. 9th edition pp 648-665. Iowa State University press. Ames, Iowa, USA.

McKeever, D.J., H.W. Reid, N.F. Inglis and A.J. Hering, 1987. A qualitative and quantitative assessment of the humoral antibody response of the sheep to orf virus infection. Vet. Microbiol., 15: 229-241.

Murphy, F.A., E.P. Gibbs, M.C. Horinek and M.J. Studdert, 1999. Paramyxoviridae In: Veterinary Virology 3rd ed. Academic Press, pp: 411-420.

Nakamura, T., Z. Lin, T. Tokuda, A. Kato, Y. Otaki, T. Nunoya and S. Ueda, 1994. Japanese IBDVS and diagnosis. Proceeding of second international symposium on infectious bursal disease (IBD) and chicken infectious anemia (CIA) 162-170 Rauischholzhausen, Germany.

Office International des Epizootics, 2001. Newcastle disease. Manual of Standards for Diagnostic Tests and Vaccines 4th edn. Paris: OIE.

Parkhurst, R.T., 1964. Pattern of mortality in avian nephrosis. Poult. Sci., 43: 788-789.

Shane, S.M., H.N. Lasher and K.W. Paxton, 1994. Economic impact of infectous bursal disease and revalence of antigenic variation for protection in infectious bursal disease. Proceeding of second International symposium on infectious bursal to disease (IBD) and chicken infectious anemia (CIA). 196-203. Rauischholzhausen, Germany.

Sharma, J.M., I-J. Kim, S. Rautenschlein and H-Y Yeh, 2000. Infectious bursal disease virus of chicken: pathogenesis and immunosuppression. Develop. Comp. Immunol., 24: 223-235.

Whitfill, C.E., E.E. Haddad, C.A., Ricks, J.K., Skeeles, L. A. Newberry, J.N., Beasly, P.D. Andrews, J.A. Thoma and P.S. Wakenell, 1995. Determination of optimum formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal antibody with IBDV. Avian Dis., 39: 687-699.

Wyeth, P.J. and N.J. Chettle, 1990. Use of infectious bursal disease vaccines in chicks with maternally derived antibodies. Vet. Rec.,126: 577-578.

Yuasa, N., T. Taniguchi, T. Noguchi and I. Yoshida, 1980. Effect of infectious bursal disease virus infection on incidence of anemia by chicken anemia agent. Avian Dis., 24: 202-209.

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