Protective efficacy of live IBD intermediate and intermedi plus vaccines against Egyptian vvIBDV challenge str (Behera /20/06)

Sultan, H.A.; H.A. Hussein ; A.G. Abd El-Razak and S.S.El Ballal
Minufiya University- Faculty of Veterinary MedicineCairo University- Faculty of Veterinary Medici

Abstract

in this study, assessment of protection of protection was carried out aga challenge with the Egyptian vvIBDV strain Behera /20/06 at 1 and 2 weeks vaccination of 49- day old commercial white –egg type chickens intermediate (Moulthroup strain) and intermediate plus (G603 strain) vaccine IBDv. Clinical signs, mortality ,gross lesions, Bursa/body weight ratio (B bursal index (BI) and histology (bursa severity index) for survivor at 7 days challenge (Pch). Follow up of maternal derived antibodies (M ,seroconversion at 7 days Pch and IBDV antigen detection in dead birds v recorded as parameters for assessment of protection. Weekly follow up of M to IBDV in chickens used in the experiment showed their absence by AGP very low level by ELISA at 49 days of age .Satisfactory seoconversion for I induced by intermediate and intermediate plus IBD vaccines were determ indicating their immunogenicity. The results of oculonasal challenge vaccinated chickens showed a partial protection at one week Pch (20% and mortalities in challenged chickens vaccinated with intermediate intermediate plus IBD vaccines, respectively) and complete protection ag mortalities was observed after two weeks Pch in chickens vaccinated with e type of IBD vaccines .the control non vaccinated and challenged chic showed mortalities between 40 to50 % at 56 and 63days of age. The b indices and histological lesions revealed that there is no complete prote against bursal atrophy or histopathological changes in bursa ,spleen and thy provided by intermediate or intermediate plus IBD vaccines at 1 or 2 weeks Although, IBD vaccines induced complete protection against mortalities weeks post vaccination, partial protection against bursal atrophy histopathological changes was observed in addition, intermediate plus vac showed some bursal damages indicating some residual pathogencity.

Introduction

Infectious bursal disease (IBD), is an acute highly contagious viral infe of young chickens described first by Cosgrove (1962) in the Delmarva a The disease leading to direct and indirect significant economic losse the world wide poultry industry (Chettle et al., 1989; Van Den Berg e 1991 and Rautenschlein et al., 2005). The direct economic losses of are due to morbidity and mortality rate while the indirect impact is d immunosuppression of infected birds (Allan et al., 1972and Ivanyi Morris, 1976).The etiological virus of the disease belongs to the rec described family Birnaviridae (Brown, 1986; Van Den Berg, 2000 Rautenschlein et al., 2003). Tow distinct serotypes I and II have identified (Jackwood and Saif 1983, and McFerran et al., 1980). Serot produces clinical disease and distinct lesions in bursa of fabricus (BF) muscular hemorrhage and serotype-2, which infected both chickens turkeys and was recorded as non-pathogenic for both species.Se

investigators, especially in the USA have reported antigenic variation among the isolates of serotype-1 IBDV. These antigenic variants were also reported through the use of a selected panel of neutralizing monoclonal antibodies (Mabs). Furthermore, in1986 very virulent (vv) strains of IBD have emerged in Europe, which can cause up to70% flock mortality in laying pullets and 100% in specific pathogen-free (SPF) chicken (Chettle et al., 1989 and Van Den Berg et al., 1991).IBD can be controlled both by live and inactivated vaccines. According to virulence, there were four kind of live serotype I vaccines: intermediate plus or hot, intermediate, mild intermediate, and attenuated mild strains. The protective efficacy of IBDV vaccines is traditionally evaluated in SPF chickens. But under field condition, residual maternal antibody (MA) levels may interfere with vaccines efficacy.

Under experimental condition, it was demonstrated that intermediate IBDV vaccines may break through residual MA and induce protective immunity, but mild vaccines not cause the disease. Over all, successful IBDV vaccination depends on the time of vaccination, the vaccine strain, the MDA status of the flock, as well as the epidemiological field isolate. (Tuskamoto et al., 1995, and Rautenschlein et al., 2005). In addition control of IBDV via adequate management and sanitation (Van Den Berg and Meulemans, 1991 and Van Den Berg, 2000), so control policy based on vaccination is considered the principle method used for control of IBD in chickens and was initially based on immunization of broilers and replacement pullets with various commercial serotype-1 live vaccines of the mild and intermediate types, and in breeder pullets either the inactivated oil-emulsion vaccines were used to boost immunity at the point of lay. Ideally, an IBD vaccine should elicit a prompt long lasting protective antibody response against virulent field strains, with lake of injury to the immune system.

Material and Methods

Chickens:

Sufficient, one-day-old commercial egg-type (L.S.L) male chicks were produced from a commercial hatchery (El-Wadi hatcheries), which possessed maternal antibodies against IBD, acquired from their parents that were vaccinated with live and inactivated oil emulsion IBDV vaccines. Chicks were monitored for IBDV-specific MDA by agar gel precipitation test (AGPT) and enzyme linked immunosorbant assay (ELISA) to determine maternal antibodies waning and the age at which the chicks become susceptible to expermental infection or vaccination.

Reference antigens and antisera:

Aknown positive and negative precipitating antigen in the form of bursal homogenates and known positive and negative precipitating reference antisera against IBDV obtained from Intervet, Inter. B. V. Boxmeer, Holland, were used for the AGPT.

IBD viruses:

a- tow types of commercial live IBDV vaccines one "intermediate" (Moulthroup strain) and one "intermediate plus" (G603 strain) vaccine obtained from the local agencies, were used in vaccination studies.

b- A local field isolate of vvIBDV designated as Behera 20/06 in the form o bursal extract was diluted 1: 10 in phosphate buffer saline, which kille 53.2% of 7-week-old susceptible commercial male chickens, was passe once in 7-week-old susceptible egg-type male chickens for propagation ar was used in vaccination studies as challenge virus.

NewCastele disease vaccines:

B-1 Type, lasota strain live ND (NewCastle disease) vaccine obtained fro the local agencies, was used in vaccination studies.

ELISA kits:

Commercial ELISA kits ProFlock supplied by Synbiotics Corporation, 11011 Frontera, San Diego. CA 92127, were used for measuring IBDV antibodie Application and interpretation of the test were carried out according to the instruction the kits manufacturers.

Samples for histopathological examination:

Bursa of Fabricius, spleen, thymus, cecal tonsils and Hadrian glands experimentally infected and control birds were fixed in neutral buffered 10 formalin solution. Tissue sections were stained with Harris hematoxyline a eosine according to Bancroft et al. (1990).

Agar gel precipitation test:

The test was used to demonstrate the presence of antibodies to IBDV examined chicken sera and for detection of IBDV antigen (s) in the cloa bursa of affected chickens as described by Wood et al. (1979).

Experimental design of determination the degree of protection and serologi response following vaccination with live intermediate (Molthroup strain) intermediate plus (G603 strain) IBD vaccines in 49- day-old commercial wh egg – type chickens and challenge with vvIBDV (Behera /20/06).

Group treatment	Vaccination 1 regime		IBD2 challenge (Age/ day)	Assessment of protection			
	age	type		Observation For days PCh7	Serology3	Antigen detection	Histopathol (SI)
Chall.vac.	49	Inter. Inter.plus	56	1-clinical signs 2-mortality % 3-Gross lesions	1-follow up of maternal derived antibodies (MDA)	Pool of bursal homogenate of dead birds	Lesion scor survivors a days PCh
Chall.non vac.	--	--	56				
Nontreated.	--	--	--				
Chall.vac.	49	Inter. Inter.plus	63	4- B:B ratio5 5-B:B index6 For survivors at 7 days PCh	2-Seroconversion at 7 days PCh		
Chall.non vac.	--	--	63				
Nontreated.	--	--	--				

1) Field dose/bird via oculonasal route

(2) The chickens were subjected to oculonasal challenge with 100ul /bird of identified local isolate Behera 20/06 in the form of bursal extract and obse

(3) Serological tests were used (AGPT& ELISA).

(4) SI=Severity index of bursal lymphoid tissue lesions (Sharma et. al., 1989).

(5) B: B ratio= Bursal body weight ratio. (Sharma et al., 1989).

(6) B: B= Bursal body weight index. (Lucio and Hitchner, 1979).

(7) PCh = Post-challenge.

Results

Decline of MDA of IBDV

Table (1) shows MDA waning of commercial white egg-type male chickens used for studying serological response and degree of protection following vaccination of IBD vaccines. The maternal precipitins were not more detectable at 35 days of age, whereas negative ELISA titers were detected at 49-day-old.

Age/days at sample collection	Serological tests			
	AGPT (Positives No./examined No.)		ELISA	
	No.	%	Titer ±Sd	%CV
7	5/5	100	16422± 497	2.579
14	4/5	80	15385± 719	3.985
21	2/5	40	11628± 3748	27.44
28	1/5	20	7825± 1966	21.823
35	0/5	0	2669± 570	18.089
42	0/5	0	1475± 500	29.203
49	0/5	0	1264±715	48.526

Table (2) shows Result of determination of degree of protection and serological response followin vaccination with live Intermediate (Molthroup) or intermediate plus (G603) IBD vaccines in 49- day old Commercial white egg – type chickens and challenge with vvIBDV (Behera /20/06).

Group treatment	Vaccination regime[1]		IBD2 challenge (Age/day)	Assessment of protection					MS
	Age	Type		Mort.[3].	B:BR[4] Mean ± sd	B:BI[5] Mean ± sd	Bursal lymphocytic tissue lesion (SI)6		
							Lymphocytic deplesion	Lymphocytic necrosis	
Chall.vac.	49	Inter Inter.plu	56	20% 10%	1.69 1.66	0.417 0.409	2.6 3.0	2.4 2.8	2.5 2.9
Chall.non vac.	--	--	56	40%	1.23	0.303	4.0	4.0	4.0
Non treated.	--	--	--	0%	4.05	1.00	0.0	0.0	o.o
Chall.vac.	49	Inter. Inter.plu	63	0% 0%	2.15 1.79	0.597 0.490	2.0 2.2	2.4 2.4	2.2 2.3
Chall.non vac.	--	--	63	50%	1.34	0.372	4.0	4.0	4.0
Non treated.	--	--	--	0%	3.6	1	0.0	0.0	0.

(1) Field dose/bird via oculonasal route

(2) The chickens were subjected to oculonasal challenge with 100ul /bird of identified local field isolate in the form of bursal extract and observed for 7 days.

(3) Mort. =mortality.

(4) B: B ratio= Bursal body weight ratio. (Sharma et al., 1989).

(5) B: B= Bursal body weight index. (Lucio and Hitchner, 1979).
(6) SI=Severity index of bursal lymphoid tissue lesions (Sharma et al., 1989).
(7) MSI=Mean severity index.

Table (3)Results of immune response following vaccination with liv Intermediate (Molthroup) or intermediate plus (G603) IBD vaccines in 49- day old Commercial white egg – type chickens and challenge with vvIBDV (Beher /20/06).

Group treatment	Vaccination regime		IBD2 challenge (Age/ day)	Serological response		
	Age	Type		AGPT (Pos. no / exam. no.)	ELISA Range	ELISA Mean ±sd
Chall.vac.	49	Inter Inter plus	56	6/8 7/9	5651-11953 10858-13984	9139±3245 12152±1214
Chall. non vac.	--	--	56	5/6	3881-11181	6731±2763
Non treated.	--	--	--	0/10	1568-2754	2438±632
Chall.vac.	49	Inter Inter plus	63	9/10 8/10	6896-14968 9477-14971	11945±3643 12574±2269
Chall.non vac.	--	--	63	5/5	4325-12146	7475±2369
Non treated.	--	--	--	0/10	886-224	1264±715

IBDV = Infectious bursal disease virus.
AGPT= Agar gel precipitation test.
ELISA = Enzyme linked immunosorbant assay

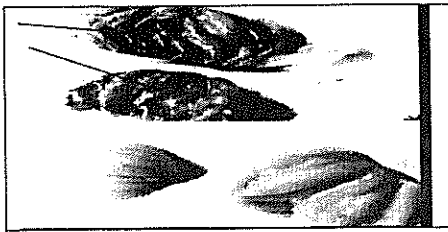

Fig (1) Hemorrhagic bursa of 49- day-old Commercial white egg – type chickens and challenge with vvIBDV (Behera /20/06

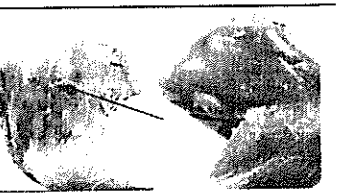

Fig (2) Hemorrhages in thigh mus of 49- day-old Commercial white e – type chickens and challen withvv IBDV (Behera /20/06).

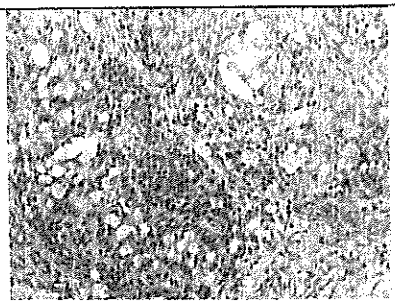

Fig (3) Bursa of 49-day- old white egg-type chicken experimentally infected with vvIBDV isolate Bherea /20/06) infected –non vaccinated control), showing degeneration of lymphoid follicles with lymphocytic depletion and cysts formation .(H&E X40)

Fig(4) Spleen of 49-day- old white egg-type chicken experimentally infected with vvIBDV isolate Bherea /20/06 (infected–non vaccinated control), showing lymphoid necrosis in germinal follicles with lymphocytic depletion and cyst formation.(H&E X40)

Fig (5) Thymus of 49-day- old white egg-type chicken experimentally infected with vvIBDV isolate Bherea /20/06 (infected –non vaccinated control), showing lymphoid necrosis and loss of lymphocyte .(H&E X10)

Discussion

The important goal of the present study focused on the control of circulating IBDV local field isolates infection by using (intermediate and intermediate plus) vaccines for this purpose, a laboratory vaccination experiments were designed to determine the development of protection to infection with IBDV local field isolate following vaccination with intermediate or intermediate plus IBDV vaccines within 7 days and 14 days PV in susceptible commercial white egg-type male chickens.

Since susceptible commercial white egg-type male chickens were difficult to obtain, maternal derived antibodies was followed up serologically, the maternal precipitins were not more detectable at 35 days of age, where as negative ELISA titers were detected at 49 days. Table (1). The results of the oculonasal challenge with local field isolates Behera/20/06 showed that there is no complete protection against mortality occurred in vaccinated groups with intermediate vaccine the mortality rate was 20 % (table 2) while it provide complete protection against the mortality after 14 PV .Also there no complete protection against mortality occurred in vaccinated group with intermediate plus vaccine and challenged with local

field isolates Behera/20/06, the mortality rate was 10 % Table (2) findings were reported by (El-Khayat, 2003 ; Abd El-Razik, 2004, and El-Aziz, 2006) these results indicating that the using of the interme plus vaccine give rapid protection against mortality than usir intermediate vaccine. So it is advisable to use intermediate plus vacc endemic area to obtain rapid protection against mortality.
Since protection against mortality might not be considered as abs criterion of efficiency of the tested vaccine other parameter refle protection against bursal atrophy were included in the experiment bursal indices and the histopathological lesions revealed that there complete protection against bursal atrophy or histological cha provided their by intermediate or intermediate plus IBD vaccines at 7 days PV. Table(2) Similar findings were reported by (Mousa et al.,19 ;Van Den Berg and Meulemans,1991 Sultan 1995 ; 1998 ;El-khayat, and Abd El-Razik, 2004). The results of the bursal indices of challe non vaccinated groups indicating that the local isolate cause sever b atrophy while in intermediate plus vaccinated and challenged gr revealed more bursal atrophy than intermediate vaccinated and challe group. similar findings reported by (Sultan, 1995 ; Bekhite et al., 1997 Abd El-Aziz, 2006)

The histopathological scoring for evaluation of the extent of bursal da Table(2) revealed that the birds challenged by local isolate showe maximum damage to bursal lymphoid tissue Table (2) and Fig (3) s findings were reported by (Helmblodet and Garner, 1964; Sultan, Cheville, 1997, and Fatma,1998) The study of histopathological lesic vaccinated groups, the results revealed that there is no com protection against histological changes provided their by intermedia intermediate plus IBD vaccines at 7 or 14 days PV. Table (2) S findings were reported by (Mousa et al., 1988-a ; Van Den Berg Meulemans, 1991 ; Bekhite et al., 1997 ; Mohammed, 1998, and Ab Razik, 2004). The histopathological examination of spleen and thym chicken experimentally infected with local isolate revealed there lymhocytic necrosis in germinal follicles and lymphocytic depletion F and 5) but less than the destructive lesion in the bursa similar finding reported by (Sharma et al., 1989; Fatma,1998; El-khayat, 2003 and El-Razik, 2004) In vaccinated groups with either intermediate or interme plus the histological examination of bursa ,spleen and thymus revealed th vaccines do not provide complete protection against bursal damage or s and thymus either after 7 and 14 day PV . From previous study It is recom to use vaccines prepared from local field isolates outbreaks after con antigenic and genetic studies to establish database for our vaccination prog .also we suggest to develop genetically engineered vaccines which can many field problems. Finally IBD vaccine development and evaluati different vaccination regimes are the main key in controlling vvIBD in Egypt

Refrences

Abd El-Aziz, A.A (2006):Studies on infectious bursal disease (gumbro)in chickens. M. V. Sci. Fac. Vet. Med. Menoufia Univ.

Abd El-Razik, A. (2004): Studies on Very Virulent Infectious Bursal Disease Virus (vvIBDV) in chickens. M. V. Sci. Thesis. Fac. Vet. Med. Menoufia Univ.

Bancroft, J.D.; A. Steven, and D.V.,Turner (1996): Theory and practice of histopathological technique 4th Ed. Churchill, Living Stone Edingburgh, London, Melbourne and New York.

Bekhit, A. B. A. ;I.A. Ghanem; H.H. Hassaine ; I. A. Ahmed, and A.G. Shakshouk (1997): Laboratory evaluation of commercial infectious bursal diseaseAvaccine against virulent infectious bursal disease virus XIth international congress of the world veternairy poultry Association. 18-22 August Budupest, Hungary .

Brown, F. (1986): The classification and nomenclature of viruses, Summary of results of meetings of the International Committee on Taxonomy of Viruses In Sendai Inter virology, 25: 141-143.

Cheville, N. F. (1967): Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen, and thymus of the chicken. Am. J. Pathol. 51 .527-551.

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

Cosgrove, A. S. (1962): An apparently new disease of chickens-Avian nephrosis. Avian Dis., 6 .385-389.

El-Khayat, F. A. (2003): Evaluation of Some Vaccination Programs against infectious bursal disease (Gumboro) in Egypt. Ph. D. Degree in Vet. Med. Sci. Thesis. Fac. Vet. Med. Tanta Univ.

Helmboldt, C. F, and E. Garner (1964): Experimentally induced Gumboro disease Avian Dis., 8: 561-575.

Ivanyi, J. and A. Morris (1976): Immunodeficiency in the chicken. IV. An immunological study of infectious bursal disease. Clin. Exp. Immunol., 23: 154-165.

Jackwood D. J. and Y. M. Saif (1983):Prevalence of antibodies to infectious bursal disease virus serotype I and II in Ohio chicken flocks . Avian Dis., 27: 850-854.

Lucio, B. and S.B. Hitchner (1979): Infectious bursal disease emulsified vaccine: Effect upon neutralizing-antibody levels in the dam and subsequent protection of the progeny. Avian Dis., 23 (2): 466-478.

McFerran, j. B.; M.S. McNulty ; E.R. McKillop ; T.J. Conner ;R. M. McCracken ; D.S. Collins, and G.M.Allan (1980): Isolation and serological studies with infectious bursal disease viruses from fowl, turkey and duck: Demonstration of a second serotype. Avian Pathol. 9:395-404.

Mohammed, A. T. (1983): A study on the pathogenesis of Gumboro disease in experimentally infected chicks. Ph.D. Thesis Fac. Vet. Med. Cairo Univ.

Mousa, S.; A. H. Bayoumi ;A. Soliman ; N.Gad, and R. Desouky (1988-a): Development of an apathogenic infectious bursal disease vaccine of turkey origin. 3rd Sci. Congo Fac. Vet. Med. Assiut Univ.

Rautenschlein, S.; H. Y.Yeh, and J.M. Sharma (2003): Comparative immunopathgenesis of mild, intermediate and virulent strains of classic infectious bursal disease virus. Avian Dis., 47(1): 66-78.

Rautenschlein S.; Ch. Kraemer; J. Vanmareke, and E. Montiel (2005): Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against vary virulrnt IBDV in commercial Broilers Avain Dis., 2005;49(2) 231-237.

Sharma, J. M.; J.E. Dohms; and A.L.Metz (1989): Comparative pathogenesis of serotype 1 isolates of infectious bursal disease virus and their effect on humoral and cellular immune competence of specific-pathogen-free chickens. Avian Dis., 33: 112-124.

Sultan, H. A. (1995): Studies on infectious bursal disease in chickens. Ph. D.Thesis. Fac. Vet. Med. Alex. Univ.

Tuskamoto, K., N. Tsukamura; S. C. Kakita; K. M. Mase; K. Imai, and H. Hihara (1995): Efficacy of three live vaccines against highly virulent infectious bursal disease virus in chickens with or without maternal antibodies. Avian Dis., (1995) 39:218-229.

Van Den Berg, T. P., and G. Meulemans (1991): Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. Avian Pathol., 20: 409-421.

Van Den Berg, T.P.; M.Gonze, and G. Meulemans (1991): Acute infectious bursal disease in poultry: Isolation and characterization of a highly virulent strain. Avian Pathol., 20 : 133-143.

Van den Berg, P. (2000): Acute infectious bursal disease in poultry: a review. Avian Pathol. 29, 175-194.

Wood, G. W.; J. C. Muskett; C. N. Hebert, and D. H. Thronton (1979): Standardization of the quantitative agar gel precipitation test for antibodies to infectious bursal disease . J. Bio1 Stand., 7 : 89-96.