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COMPARISON OF THE EFFICACY OF SOME REGIMES OF VACCINATION FOR THE CONTROL OF VVIBDV IN BROILERS

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ABSTRACT

Two vaccination programs for infectious viral disease (IBD) were compared in broiler chicken with maternal immunity, placed in tow farms, A and B. IBD immune complex vaccine was administered by subcutaneous route, at the hatchery into chicks of farm A at age of 1 day. On farm B, the attenuated intermediate plus live IBD vaccine was given via drinking water at the age of 16th day. The vaccine uptake was monitored via serology , bursal indices and effect of the two vaccines on immune response to Newcastle disease and avian influenza vaccines. It was also verified by an experimental very virulent IBD challenge performed at the age of 28th day in birds transferred from the farms with appropriate control groups in a laboratory. The immune response on farm A showing higher serological titer at the age of 35 day via ELISA test. Both two vaccines provide complete protection against mortality but not protection against bursal atrophy or histopathological changes after challenge. As well as RT-PCR-based viral IBD detection in bursa of Fabricius at 7th day post challenge.

Keywords: Infectious *bursal disease virus* (IBDV), IBD immune complex vaccine, ELISA, RT-PCR.

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INTRODUCTION

Infectious *bursal disease virus* (IBDV), a member of the genus Avibirnavirus of the family *Birnaviridae* is the causative agent of infectious bursal disease (**Giambrone *et al.*, 1977**), a highly contagious immunosuppressive disease which cause sever economic losses in commercial broiler production (**Balamurugan and Kataria, 2006; Thierry and Van Den, 2000**). IBDV can induce severe immunosuppression in susceptible chickens which fail to respond optimally to routinely used poultry vaccines (**Sharma, 1986**). Commercial chickens are usually infected early in life so it necessary to establish protection as early as possible.

During the last decade, very virulent IBDV has caused outbreaks of disease with high mortality in Europe and other parts in the world. In Egypt (in the summer of 1989), severe outbreaks of very virulent IBD (vvIBDV), similar to those reported in European countries in both vaccinated and non-vaccinated flocks, and were associated with high mortalities up to 70% in replacement layer pullets and 30% in meat-type birds (**El-Batrawi, 1990; Ahmed, 1991 and 1993 and Khafagy *et al.*, 1991**). The incidence of IBD virus infection and its associated disease problems were still common in Egypt in spite of the routinely applied vaccination program (**Sultan, 1995; Elham El-Ebiary *et al.*, 2001; Nadia, *et al.*, 2001; Hassan *et al.*, 2002; Abdel-Alim *et al.*, 2003; Abd El-Razak, 2004 and Metwally *et al.*, 2008**). It is noticed that the current vaccination programs failed to protect chicks sufficiently. Vaccination failure mainly is due to the inability of the intermediate vaccines to protect the birds before they become susceptible to challenge with virulent field virus. However, when progeny are vaccinated at an early age with mild or highly attenuated live vaccine, high level of maternal derived antibodies (MDA) may interfere with the development of active immunity (**Skelees *et al.*, 1979; Van Den Berg and Meulemans, 1991**). Because of MDA interference associated with lack of uniform antibody titers in the progeny (**Winterfield and Thacker, 1978**). Repeated vaccinations are needed until MDA wanes (**Clotti *et al.*, 2001**).

Recently, an immune complex IBD vaccine (IBDV-Icx)

was developed for in ovo immunization consisting of IBDV-specific antibodies mixed with a live, attenuated IBDV strain (2512-IBDV) with intermediate plus virulence (**Whitfill *et al.*, 1995**). The reason to mix antibodies with intermediate plus IBDV vaccines to form complex vaccine is to reduce the virulence of the IBD-viral strain used when applied in ovo or to young chickens (**Whitfill *et al.*, 1992**). The second reason is to stimulate an early immunoresponse in chickens when they have high level of MDA (**Clotti *et al.*, 2001**).

The present study aimed to compare the efficacy of two IBD vaccination programs using two types of IBD vaccines in broilers raised in the field in the presence of maternal derived antibodies.

MATERIALS & METHODS

Farms

The field trial took place in commercial facilities of poultry area. The trial included 2 houses in to 2 different sites to avoid cross contamination (farm A, farm B).They were populated at the same day of Hubbard breed each capacity of house is 50.000 birds **(Table 1).**

Table 1. Vaccination program of Farm A and Farm B

Age/day	Farm A	Farm B
1	Immune complex vaccine S/C	--
7	Hitchner-IB	
10	AIV killed vaccines: subcutaneous injection of 0.5 ml	
16	--	Intermediate plus vaccine via drinking water
18	Lasota via drinking water	
27	Lasota via drinking water	

Experimental birds

100 broiler one day-old chicks of the same farm type (Hubbard) were used. The chicks were floor reared under natural day light in strictly isolated experimental rooms, previously cleaned and disinfected and were provided with commercial broiler starter ration. Water and feed were provided adlibtum.. The experimental birds were divided into two groups, 50 birds for each group. One group was vaccinated against Newcastle and avian influenza as in the two farms, while the other group did not receive any vaccine and used for maternal antibodies waning .

Agar gel precipitation test (AGPT)

A known 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. IBDV antigen was prepared according to **(Hirari and Shimakura, 1972)**.

Haemagglutination (HA) and haemagglutination inhibition (HI) tests:

Procedures were performed according to **OIE Terrestrial manual (2009)**. Known positive and negative NDV and AIV antisera were obtained from Intervet international/B.V. Boxmeer, Holland.

IBD Enzyme Linked Immunosorbant Assay (ELISA) kits for antibodies detection:

Commercial ELISA kits ProFlock supplied by Synbiotics Corporation, 11011 via Frontera, San Diego. CA 92127.

IBD vaccines and challenge virus:

a- One type of commercial live IBDV vaccines "intermediate plus" (G603 strain) vaccine obtained from the local agencies, were used in vaccination studies.

b-An immune complex vaccine that contains specific antiserum mixed in the appropriate ratio with intermediate plus (winterfield 2512) IBD vaccine strain. obtained from the local agencies, were used in vaccination studies

c- Challenge IBDV is a local field isolate of vvIBDV in the form of bursal extract was diluted 1: 10 in phosphate buffer saline.

The experimental challenge was conducted in faculty of veterinary medicine, Menofia University **(Table 2).**

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR amplifications were carried out on bursas from vaccinated and 7 days post-challenged birds. PCR products from positive samples were sequenced. Phylogentic analysis was performed on the obtained sequences. RT-PCR amplifications, sequencing, and phylogenetic analysis were conducted in CEVA Phylaxia ZRT Szallas utcas1107-Budapest-Hungary.(Quality control department).

Histopathological examination

Specimens of the bursas, was fixed in 10% formalin solution. Tissue sections were stained with Harris hematoxyline and eosine according to (**Bancroft *et al.*, 1996**). The severity of bursal lymphoid tissue lesions were scored from 0 to 4 on the basis of lymphoid necrosis and/or lymphocytic depletion according to **Sharma *et al.*, (1989)** as follows:

0= less than 5% of the lymphoid follicles (per field) affected,

1= 5-25% of the lymphoid follicles (per field) affected.

2= 25-50% of the lymphoid follicles (per field) affected.

3= 50-75% of the lymphoid follicles (per field) affected.

4= More than 75% of the lymphoid follicles (per field) affected

Table 2. Experimental design of determination of the study

1) Field dose/bird via oculonasal route 2) challenge with 100 ul/bird of local field isolate via oculonasal route 3) serological tests used (AGPT &ELISA) 4) SI: Severity index of bursal lymphoid tissues 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.

Group treatment	Vaccination [1] regime		IBD[2] challenge (Age/ day)	Assessment of protection				
	age	type		Observation For days PCh[7]	Serology[3]	Antigen detection	Histopathology4 (SI)	Virus detection
Vaccinated challanged	1 16	IBDV-Icx Inter. plus	28 28	clinical signs mortality gross lesions B:B ratio B:B index for survivors at 7 days PCh	*follow up of maternal derived antibodies (MDA) *Seroconversion at 7 days PCh	Pool of bursal homogenate of dead birds	Lesion score for survivors at 7 days PCh	PCR 7 days PCh
Vaccinated non challenged	1 16	IBDV-Icx Inter. plus	-					
Non vaccinated challenged	-	-	28					
Non vaccinated non challenged	-	-	-					

RESULTS & DISCUSSION

Since 1987, acute IBD cause up to 30-60 % mortality in broiler and pullet flocks, respectively. IBD outbreaks with these characters appeared in Egypt and occurred since 1989 and have caused serious economic losses despite vaccination **(El-Batrawi, 1990; Khafagy *et al.*, 1991; Sultan, 1995; El-Khayat, 2003)**.This study aimed to compare the efficacy of two IBD vaccination programs using two types of IBD vaccines in broilers raised in the field in the presence of maternal derived antibodies (MDA) IBDV passive immunity. The field observation at the farms level illustrated the difficulties in managing a vaccine programs with attenuated intermediate vaccines in the presence of MDA. So two vaccination programs were designed, farm A received IBDV-Icx at first day of age by S/c administration, while farm B received intermediate plus vaccine at 16th day of age via drinking water. The comparison between the two vaccines included the serological response (measured by ELISA and AGPT), the bursal index, the effect on immunoresponse to live NDV vaccine and inactivated AI vaccine and protection against vvIBDV at 28th of age.

Firstly the sera obtained from the two farm A and farm B, at 7, 14 , 21,28 and 35 day of age and tested for IBD antibodies by ELISA and AGPT. Decline of MDA to IBDV was seen during the first few weeks **(Figure 1)** while at 3rd weeks there is active humeral immunoresponse demonstrated by increase of the antibodies titers to IBDV ,The active immunoresponse begin at 21st day of age in farm A (IBDV-Icx vaccinated) and 28th day of age in farm B (intermediate plus vaccinated) while in non vaccinated controls the decay of MDA to IBDV continued and chickens having lower MDA. **Sharma (1985); Van den berg and meulemans (1991)** reported that Maternal IBDV antibodies interferes with protection from vaccinal virus. additionally, IBDV-Icx is not neutralized by Maternal IBDV antibodies **(Van Den Wijnagaard *et al.*, 2001)** The result (**Figure 1**) also showed the serological response of farm A

(IBDV-Icx) was higher than of farm B (intermediate plus vaccinated), that indicates the ability of transmune IBD vaccine to withstand the neutralizing effect of MDA.

Secondly, the bursal indices of farm A and farm B was measured at 7th ,14th ,21st,28th and 35th day of age according to **Lucio and Hitchner (1979)**, The results showed that the mean of B.B index of vaccinated was not different of non vaccinated birds at 14th day of age that indicating that the maternal antibodies still high enough to prevent the replication of vaccine virus .By 3rd week of age the mean of B.B indecies of IBDV-Icx group (Farm A) were 0.79, 0.69 and 0.59 at 21st, 28th and 35th day of age respectively and was 0.65 and 0.55 at 28th and 35th day of age in intermediate plus group (Farm B) **(Figure 2)** that indicates the both vaccines bursal atrophy similar findings were reported by (**Chansiripornchai and Sasipreeyajan, 2008**).

Thirdly, Newcastle and avian influenza are highly contagious diseases causing serious problems to poultry industry due to they causing high mortality rates to affected chickens. The control of such diseases depend on vaccination so it is very important to study the effect of " IBDV-Icx ", or "intermediate plus" IBDV vaccines in broiler chickens on immunoresponse to live Newcastle virus vaccine and inactivated oil emulsion avian influenza vaccine. as shown in **(Figure 3&4)** the maternal antibody waning of broiler chickens used for studying the immunoresponse of ND and AI vaccines on chickens after vaccination with IBDV-Icx or intermediate plus vaccines the geometric mean (GM) of HI titers log2 of NDV was (0) at 28 days of age and was (0.2) of AI at 35 days of age. **(Figure 3&4)** showed the determination of serological response of live ND vaccine and oil emulsion AI vaccine in broiler chickens after vaccination with IBDV-Icx or intermediate plus IBDV vaccines, the results revealed that the IBDV vaccines either IBDV-Icx or intermediate plus have immunosuppressive effect on immunoresponse to live Newcastle virus vaccine and inactivated oil emulsion avian influenza vaccine that may due to

the destructive effect on bursa of Fabricius which the main source of B-lymphocyte responsible for humeral antibodies. **Rautenschlein *et al.* (2007)** compared immunosuppressive abilities prosperities of different IBDV live vaccines (intermediate, intermediate plus in specific-pathogen-free (SPF) layer type chickens or commercial broiler. The Newcastle virus (NDV) vaccination model was applied to determine not only IBDV-induced immunosuppression but also bilateral effects between IBDV and NDV. None of IBDV vaccine abrogated NDV vaccine-induced protection. All NDV-vaccinated SPF layer and broilers were protected against NDV challenge independent on circulating NDV antibody level. Sustained suppression of NDV antibody development was observed a temporary suppression of NDV antibody development was observed in SPF layers, which had received the intermediate plus IBDV vaccine . also a temporary suppression of NDV antibody development in broilers vaccinated with one of the intermediate, as well as the intermediate plus IBDV vaccines.

The important aim of the present study focused on the control of circulating vvIBDV local field isolate infection by using IBDV-Icx or intermediate plus vaccine. Chickens from each farm were challenged at 28th day of age ,the result of oculonasal challenge showed that IBDV-Icx or intermediate plus vaccine provide complete protection against mortality . Similar findings were reported by **(Sultan, 1995; Bekhite *et al.*, 1997 and Abd El-Razak, 2004)** they mentioned that he intermediate plus can provide complete protection against mortality ,While **(payla *et al.*, 2003)** reported that the immune complex IBD vaccine can provide complete protection against mortality after challenge with vvIBDV. While the mortality rate was 5% in non vaccinated group, The gross lesions were mainly dehydration, extensive hemorrhage on the muscles of the thigh and breast and the BF was constantly involved and was enlarged, edematous, hemorrhagic and contained blood in the lumen. Similar findings was reported by **Khafagy *et al.* (1991) and Sultan (1995)**.

Since protection against mortality might not be considered as absolute criterion of efficiency of the tested vaccine other parameter reflecting protection against bursal atrophy were included in the experiment, the bursal indices and the histopathological lesions revealed that there is no complete protection against bursal atrophy or histological changes provided ether by IBDV-Icx or intermediate plus IBD vaccine at 7 days post challenge . **(Table 3 & 4)** Similar findings were reported by **(Sultan, 1995 ; Bekhite *et al.*, 1997 ; Payla *et al* 2003, and Abd El-Razik, 2004)**. As observed the IBDV antibody complex vaccine was shown to be effective in the field trials and to be protective, and also high level of maternal antibodies did not interfere with the IBDV-Icx ability to immunize broilers. RT/PCR was extensively used and considered the most rapid, sensitive and accurate method for detecting IBDV. The PCR eliminates the need to grow or isolate IBDV before amplification . **(Jackwood and Jackwood, 1997; Majo *et al.*, 2002)**. RT/PCR used amplify the VP2 gene **(Jackwood and ackwood, 1994; Jackwood and Jackwood, 1997;)**. The hypervariable region of VP2 gene was the target fragment to be rapidly sequence most of IBDV field strains **(Ibrahim, 2000 and Abdel-Alim *et al.*, 2003)**.

The vaccinated and challenged groups showed PCR bands at the expected size (408 bp) **(Figure 5)**. Sequence analysis confirmed that these bands were from hypervariable region of vp2 gene of IBDV. Phylogenetic analysis showed that the filed strain that was used in challenge trails is a very virulent strain **(Figure 6)**.

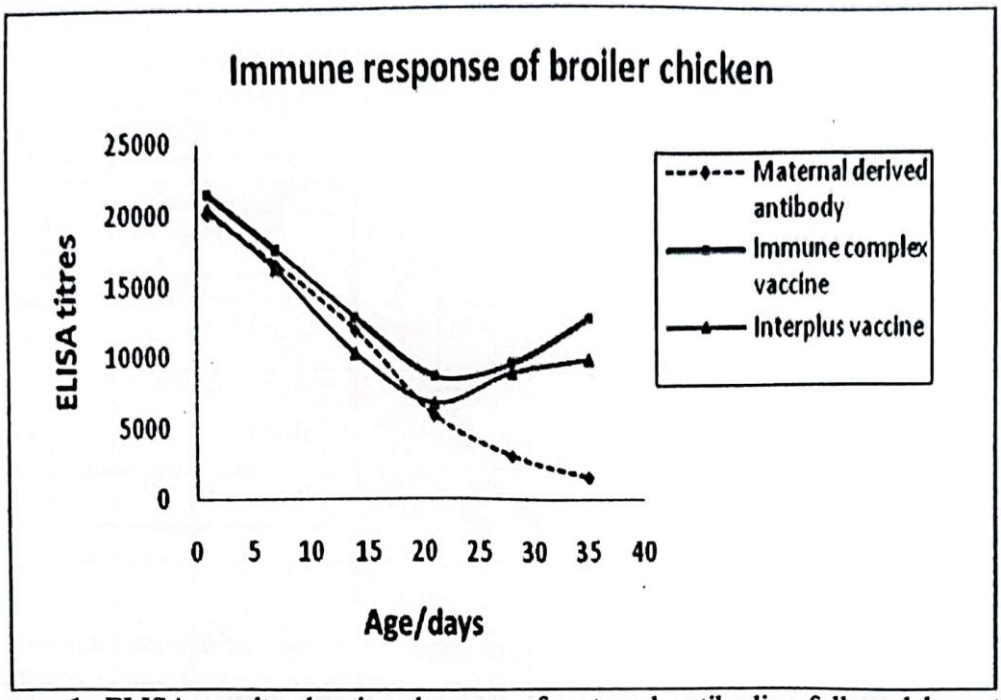

Figure 1. ELISA results showing decrease of maternal antibodies followed by active immune conversion. Farm A received immune complex vaccine and farm B intermediate plus vaccine. The average titer is shown for each farm and sampling time. Farm A showing higher immune response than farm B.

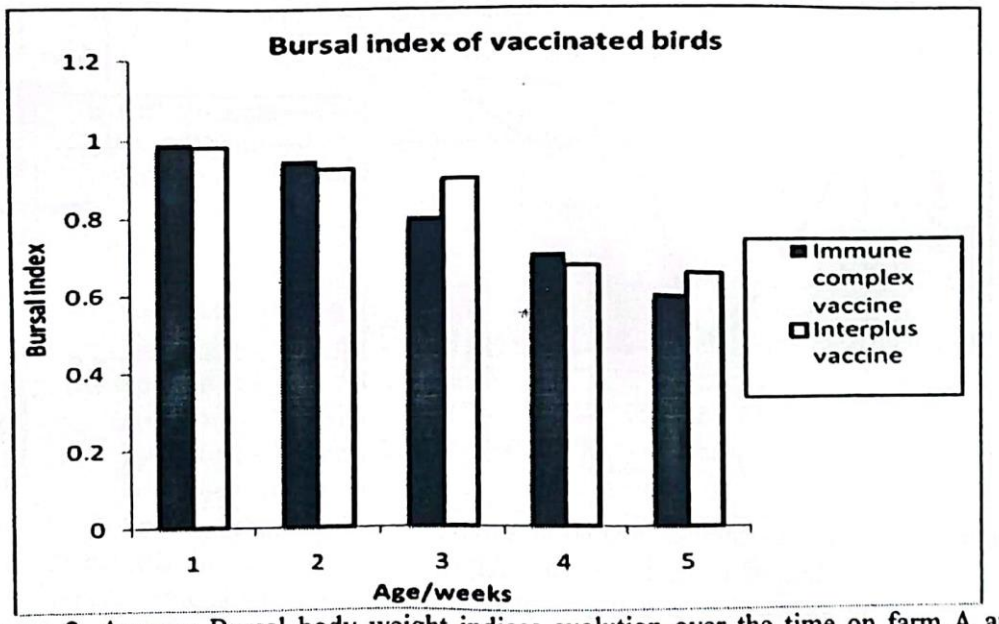

Figure 2. Average Bursal body weight indices evolution over the time on farm A and farm B.The results showing both vaccines causing bursal atrophy

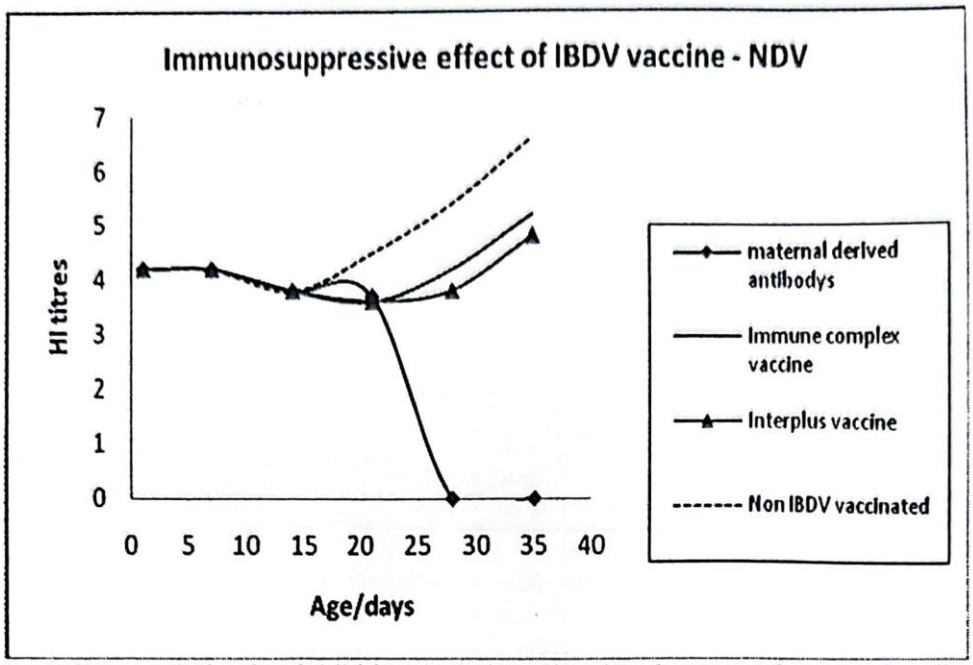

Figure 3. Hemagglutination inhibition (HI) titer showing decrease of maternal antibodies of Newcastle disease (ND) and HI of farm A received immune complex vaccine and arm B intermediate plus vaccine is lower than of non IBDV vaccine received birds reared in laboratory.

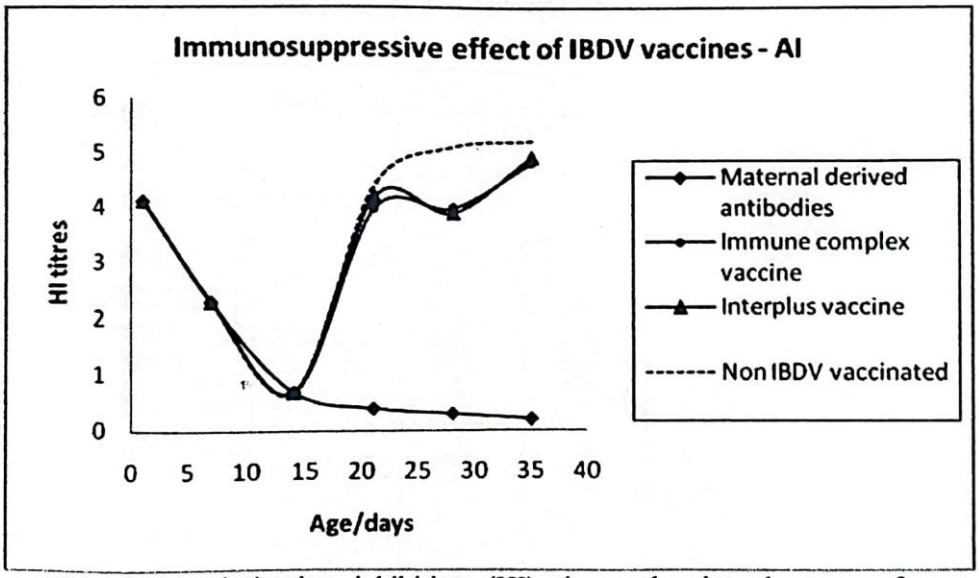

Figure 4. Hemagglutination inhibition (HI) titer showing decrease of maternal antibodies of avian influenza (AI) and HI of farm A received immune complex vaccine and arm B intermediate plus vaccine is lower than of non IBDV vaccine received birds reared in laboratory.

Table 3. Results of the degree of protection of the broiler chickens following vaccination with immune complex vaccine (IBDV-Icx) or intermediate plus IBDV- vaccines and challenge at 28- day of age with vvIBDV.

Group treatment	Vaccination [1] regime		IBD[2] chall. (Age / day)	Assessment of protection					
				Mort. %	B:B[5] ratio	B:B [6] index	Bursal lymphocytic lesion		MSI[4]
	Age	Type					Lymphc. depletion	Lymphoc. necrosis	
Vaccinated Challenged	1	IBDV-Icx	28	0%	0.95	0.40	3.6	3.4	3.5
	16	Interm. Plus		0%	0.88	0.37	3.6	3.2	3.4
Vaccinated Non Challenged.	1	IBDV-Icx	–	0%	1.46	0.62	3.4	3.2	3.3
	16	Interm. plus		0%	1.35	0.58	3.2	3.0	3.1
Non vaccinated Challenged	--	--	28	5%	0.69	0.29	4.0	4.0	4.0
Non vaccinated non challenged	--	--	--	0%	2.32	1.0	0.0	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

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

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

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

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

Table 4. Results of serological response following subsequent ocular vaccination with immune complex vaccine (IBDV-Icx)or intermediate plus IBDV vaccines and challenge at 28 -day of age with vvIBDV.

Group treatment	Vaccination regime		IBDV chall. Age/day	Serological response		Virus detection PCR
	Age	Type		AGPT (Pos. no./ exam. no)	ELISA Mean ± sd	
Vaccinated Challenged	1 16	IBDV-Icx Interm. Plus	28	8/10 7/10	10326±1164 11055±2236	+ve +ve
Vaccinated Non Challenged.	1 16	IBDV-Icx Interm. plus	--	2/10 1/10	9231±3774 6453±2189	Not done Not done
Non vaccinated Challenged	--	--	28	8/9	13564±985	+ve
Non treated	--	--	--	0/10	3254±1543	-ve

IBDV= Infectious bursal disease virus.
ELISA= Enzyme linked immunosorbant assay.
Sd= standard deviation.
CV =Coefficient of variant.

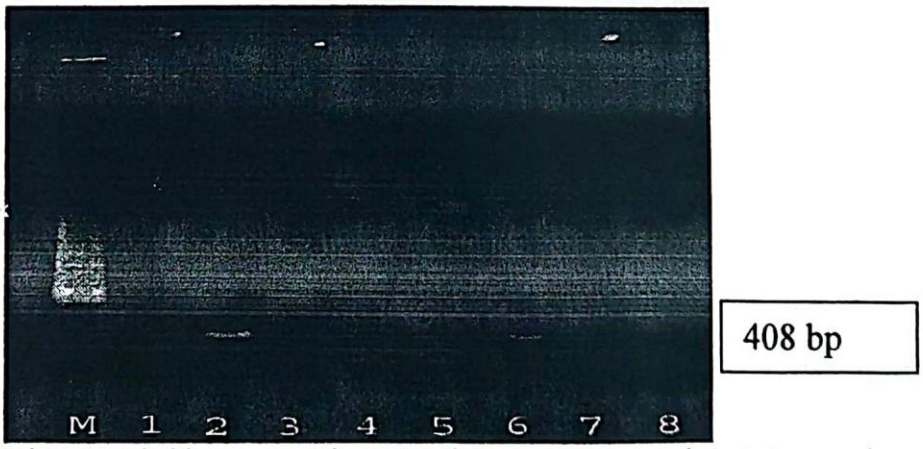

Figure 5. M: 100bp DNA ladder, 1: negative control, 2: positive control, 3, 5, 7: negative samples, 4: immune complex vaccinated and challenged group, 6: intermediate plus vaccinated and challenged group, 8: non vaccinated challenged groups.

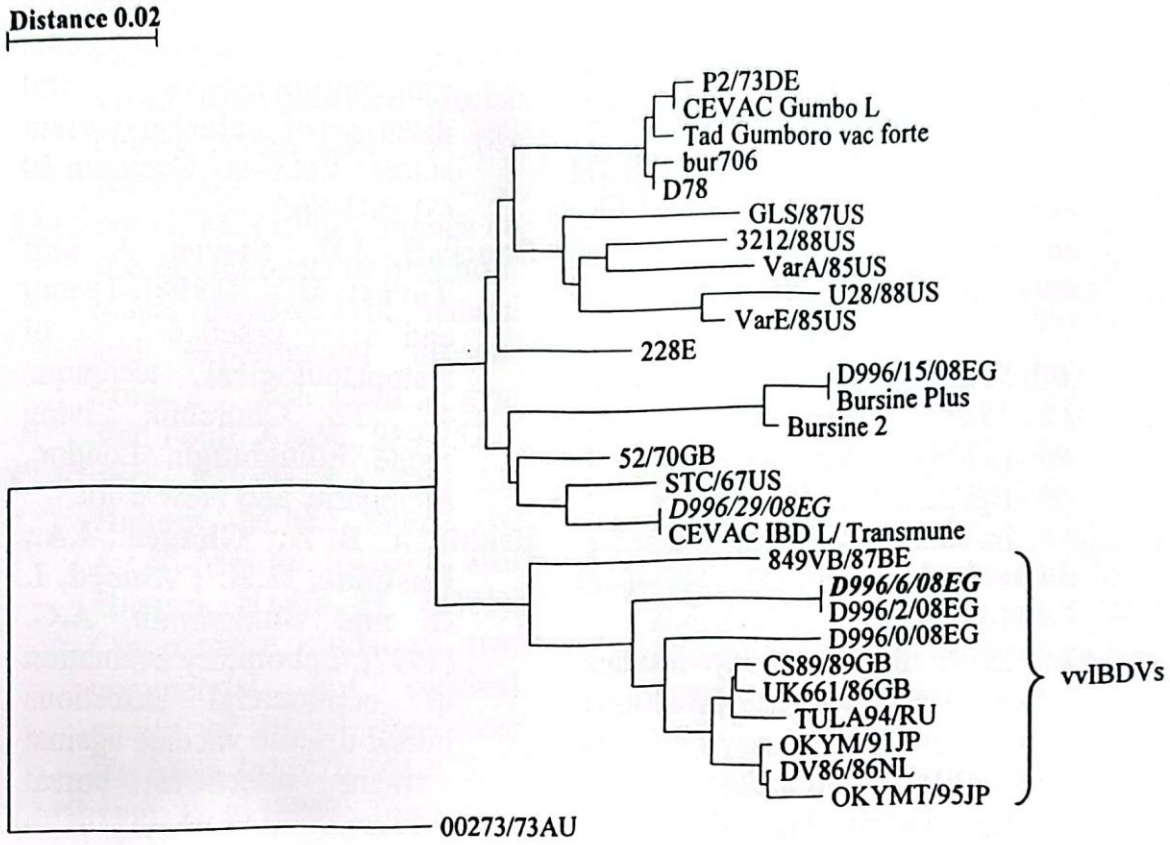

Figure 6. Phylogenetic analysis was carried out based on the 408 base pairs long (721-1128 bp) nucleotide sequence of the hypervariable region of the vp2 gene of IBDV. Bold and italic sequences code indicated the obtained sequence in the study. **D996/6/08EG** is the nucleotide sequences isolated from all challenged groups with field strain.

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