Evaluation Of Chickens Immune Response Post Vaccination With Some Viral Vaccines

Hegazy A M* and Abd El Samie L K**

*Prof. and Head of Avian and Rabbit Medicine, Fac.of Vet. Med., Zagazig University

**Veterinarian and Director of the Mobile Vet. Unint, Fac. of Vet. Med., Zagazig Univ.

ABSTRACT

The immune response of chickens was evaluated after vaccination with bivalent and trivalent viral vaccines by using Hemagglutination Inhibition, Enzyme Linked Immunosorbent Assay and challenge tests. One hundred and sixty day-old chicks (Arbor Acre plus) were divided into 4 groups, three groups were vaccinated with different viral bivalent or trivalent vaccines against Newcastle disease, Infectious bronchitis and Infectious bursal disease. The fourth group of chickens remained as negative control group without vaccination. The results of immune response monitoring at 3 and 4 weeks post vaccination, showed decline of antibody titre in chickens vaccinated with live bivalent (ND+IB) vaccine at 9 days of age, while revaccinated chickens with the same vaccine at 14 days-old revealed higher antibody titre which increased at 4 weeks post vaccination. Chickens vaccinated with bivalent inactivated vaccine (ND+IBD) at 10 days-old showed lower antibody titre than chickens revaccinated with the same vaccine at 22 days. The highest level of antibody titre was recorded in chickens primed and boosted with trivalent inactivated vaccine (ND+IB+IBD), the protection percent was 100% post challenge.

INTRODUCTION

Immunization against infectious diseases has proven to be one of the most cost-effective methods of the controlling economic losses in livestock and poultry *(1)*. Newcastle disease virus (NDV) is a widespread and economically important poultry pathogen. Although vaccines have long been available and administered to control Newcastle disease, the virus remains an ongoing threat to commercial flocks *(2)*. Infectious bursal disease (IBD) is an acute and highly contagious disease of young chicken caused by Birna virus. Mortality of infected birds can be prevented if injected with antibodies *(3)*. Infectious bronchitis (IB) is a highly contagious disease that affects not only young chicks and broilers but also the birds in lay *(4)*. Control of those infections by vaccination is highly successful in chickens. An oil emulsion trivalent vaccine was prepared from NDV, IBDV and IBV antigens to investigate its immunological efficacy in chicks against each antigen. The immunogenicity against these diseases was proved *(5)*.

MATERIAL AND METHODS

I. Materials

1. **Experimental chickens:** One hundred and sixty normal day-old chicks (Arbor Acre plus), obtained from El-Banna Poultry Company hatchery (Egypt) were used in this study.
2. **Vaccines:** Monovalent, bivalent or trivalent vaccines were kindly supplied by Intervet Schering Plough Co.(Egypt).

2.1.Monovalent live vaccines

- Nobilis ND Hitchner: contains 6.0 $\log_{10}$ ELD_{50} of ND virus Hitchner B1/dose. Batch No. 080586C
- Nobilis IB H120: contains 3.0 $\log_{10}$ EID_{50} of strain H120 type Massachusetts/dose. Batch No. 080577C
- Nobilis Gumboro D78: contains 4.0 $\log_{10}$ TCID50 of Gumboro strain D78/dose. Batch No. 080580C.

2.2.Bivalent vaccines

- Nobilis MA5 + Clone 30 (live vaccine): contain 10^3 EID50 IB virus strain MA5 and 10^6 EID_{50} ND virus stain Clone 30/dose. Batch No. 080589C.

- Nobilis G+ND (inactivated in oil adjuvant emulsion vaccine): contains 30 ≥ 50 PD_{50} or inducing 4 log_2 HI units per 1/50 of ND virus Clone and Gumboro virus strain D78 inducing 14.5 log_2 VN units/dose. Batch No. A567A02

2.3.Trivalent vaccines

- **Nobilis IB+G+ND** (inactivated in oil adjuvant emulsion vaccine): contains IB strain M41 inducing ≥ 6.0 log_2 HI units, 30 ≥ 50 PD_{50} or inducing 4 log_2 HI units per 1/50 of ND virus Clone and Gumboro virus strain D78 inducing 14.5 log_2 VN units/dose. Batch No. A592A01

3. **Viral strains for challenge:** All viral strains were provided by the Department of Avian and Rabbit Medicine, Faculty of Vet Med., Zagazig University.

- *Newcastle disease virus (NDv).*
- *Infectious bursal disease virus (IBDv).*

4.**Embryonated chicken eggs:** 450 (ECE) for propagation and titration of viral strains. Embryonated chicken eggs (ECE) 9-11 days were inoculated, 5 embryos for each virus dilution via allantoic cavity (AC) for NDv and chorio-allantoic membrane (CAM) for IBDv.

5.ELISA Commercial kits:

- ND Antibody Test Kit: BioChek B.V., Crabethstraat 38-C, 2801 AN Gouda, Holland. Batch No. A07-KB-04/04-CK116.
- CIVTEST AVI IBV: LSI - 1 bis Allée de la Combe – 69380 LISSIEU. Batch No.3F6Q.
- IBD Antibody Test Kit: BioChek (UK) Ltd., 11Mill farm Business Park, Millfield Road, Hounslow, London. Lot No. FS4729-CK113.

Also prepared ELISA kits for detection of ND, IB and IBD, antibodies at different serum dilutions (from 1:100 to 1:3200) were used and performed at the Bio-Technology Laboratory, Faculty of Veterinary Medicine, Cairo University.

6. Serum samples: Sera were harvested and stored at -20°C until used.

II. METHODS

1. Preparation of antigen for ELISA:

NDV, IBV and IBDV antigens were prepared after ***9, 8*** and ***7*** respectively.

2.Hemagglutination (HA) and Hemagglutination Inhibition (HI) tests

HI test was applied by using 4HAU ***(6).***

3.Enzyme Linked Immunosorbent Assay (ELISA)

ELISA for IBD ***(7),*** ELISA for IB ***(8),*** and ELISA for ND ***(9)*** were carried out.

Commercial ELISA (1:500) was performed according to the manufacturer instructions in the Central Lab., Faculty of Vet. Med., Zagazig University.

4. **Preparation of challenge viruses:** Both NDV and IBDV were propagated and titred to contain 10^6 EID_{50}/ml and 104 EID_{50} /ml respectively, for challenge test.

5. **Experimental design:** One hundred and sixty day-old chicks were divided into four groups, three groups were vaccinated with different types of vaccines and the fourth group was control unvaccinated (Table 1).

Table 1. Experimental Design.

Group	Subgrp	No. of birds	Type of vaccine						Evaluation tests			
			Bivalent	Trivalent	Live	Killed	Age/day	Route	HI	$ELISA_1$	$ELISA_2$	challenge
1	1A	10	ND+IB	---	1 dose	---	9	Eye drops	+ve	+ve	+ve	+ve ND
	1B	10	ND+IB	---	2 doses	---	day old	Eye drop	+ve	+ve	+ve	+ve ND
							14					
2	2A	15	ND+IBD	---	---	1 dose	10	0.5ml S/c	+ve	+ve	+ve	+ve ND, IBD
	2B	15	ND+IBD	---	---	2 doses	day old	0.5ml S/c	+ve	+ve	+ve	+ve ND, IBD
							22	0.5ml S/c				
3	3A	15	---	ND+IB+IBD	1 dose each	1 dose	1,7,10*	Eye drop	+ve	+ve	+ve	+ve ND, IBD
							22 d.o.	0.5ml S/c				
	3B	15	---	ND+IB+IBD	1 dose each	2 doses	1,7,10*	Eye drop	+ve	+ve	+ve	+ve ND, IBD
							14 d.o.	0.5ml S/c				
							33 d.o.	0.5ml S/c				
4		80	Unvaccinated						+ve	+ve	+ve	+ve ND, IBD

HI: Haemagglutination Inhibition test.
$ELISA_1$: Enzyme-linked Immunosorbent Assay with single dilution of serum (commercial kits).
$ELISA_2$: Enzyme-linked Immunosorbent Assay with different dilution of serum.
*age of priming with live vaccine

RESULTS AND DISCUSSION

By single dilution ELISA (1:500), 3 weeks post vaccination with live bivalent ND+IB vaccine, intra-ocular (I/O), chickens showed the lowest antibody titre 1543 for ND and 659 for IB, moreover the titre declined 4 weeks post vaccination to 1198 for ND and 521 for IB. Higher antibody titre was obtained post revaccination with the same vaccine 2530 and 1755 for ND and IB, respectively, 3 weeks post vaccination, while the titre increased 4 weeks post vaccination to 2586 and 1948 for ND and IB, respectively. Meanwhile vaccination with bivalent inactivated ND+IBD vaccine subcutaneously (S/C), the antibody titres for ND and IBD were 2523 and 1878 respectively, 3 weeks post vaccination with slight increase post 4weeks 2543 and 1900 respectively. Revaccinated chickens with the same vaccine at 22 days-old via the same route, the antibody titre range was higher for ND and IBD 2815 and 2231 respectively, 3 weeks post vaccination and rose to 2878 and 2448 respectively, 4 weeks post vaccination. Unvaccinated control group showed negative antibody titre.

The highest antibody titre was recorded in chickens vaccinated primly with live vaccines (I/O) then boosted with two doses of trivalent ND+IB+IBD inactivated vaccine (S/C) at 14 and 33 days-old, where the antibody titre 3 weeks post vaccination for ND, IB and IBD was 3166, 3696 and 3197 respectively, then rose 4 weeks post vaccination to 3331, 4138 and 3508 respectively. While chickens primed with live vaccine (I/O) and boosted with one dose of trivalent ND+IB+IBD inactivated vaccine (S/C), the antibody titre was 2738, 1776 and 2213 three weeks post vaccination, then raised to 2801, 3050 and 2321 four weeks post vaccination for ND, IB and IBD, respectively, (Table 2). Birds initially received the live vaccine and inactivated vaccines, developed higher antibody titres to both ND and IBD viruses, moreover birds received the combination live and inactivated vaccine subcutaneously (S/C), had the highest antibody titer ***(10)***, it has been recorded that sixty commercial hens (63 weeks old) injected I/M with an inactivated combination NDv, IBv and IBDv vaccine after routine vaccination with modified live vaccines for each disease, the mean antibody titre by ELISA were 7288, 4970 and 4902 for IBDv, IBv and NDv respectively at 6 weeks post vaccination ***(11)*** and by using ELISA, it was proved that the subcutaneous or wing muscle routes of injection of combined ND/IB inactivated vaccine resulted in higher antibody titres to NDv through 16 weeks post injection; however no differences were found in antibody titres to IBV among the various groups due to site of injection ***(12)***. Moreover, inactivated oil-emulsion ND vaccine containing Colne30 induced the highest antibody titre in the serum and the antibody titre obtained 3-6 weeks after vaccination with different doses of the vaccines remained approximately constant until 18 weeks after vaccination ***(13)***, and chickens in the groups primed with P/VP243/E and boosted with killed IBD vaccine had higher ELISA titres $(P<0.05)$ than chickens in control group or groups only primed with P/VP243/E ***(14)***.

By different dilutions ELISA, Three weeks post vaccination with live bivalent (ND+IB) vaccine, the mean optical density (OD) for ND and IB values were positive at 4 dilutions only and after 4 weeks, only 3 dilutions were positive. While 3 and 4 weeks post revaccination with the same vaccine, all serum dilutions were positive (Table 3, 4). ELISA, HI and NT were used for detection of IBV recording that inoculation with live IB vaccine (H120) induced highest titres within 21 days and after this time titres declined towards 63rd day and the ELISA absorbance value compared to the HI value at different dilution of serum showed a significant correlation ($p \leq 0.01$) between both tests ***(15)***, serum IBV enzyme-linked immunosorbent assay antibody geometric mean titers (GMTs) after vaccination with the combinations of live attenuated strains were low, ranging from 184 to 1,354, prior to IBV challenge at 10 wk of age, while both inactivated vaccines induced an anamnestic response of similar magnitudes with serum GMTs of 6,232-12,241 ***(16)***. Eye drop method for live ND vaccine application induced high immune responses, at 3 weeks post vaccination higher percentage positivity, 96 (3.5 ± 0.49) by ELISA which declined to

Table 2. Immune response post vaccination by using ELISA at single serum dilution (1:500)

group	subgrp	No. of birds	Vaccine				3 weeks post vaccination									4 weeks post vaccination								
							Mean OD at 1:500			S/P value			Titre range			Mean OD at 1:500			S/P value			Titre range		
			Type	V/D	Route	Age/ day	IB	IBD	ND	IB	IBD	ND	IB	IBD	ND	IB	IBD	ND	IB	IBD	ND	IB	IBD	ND
1	A	10	Live	*	ye dro	9	0.332		0.443	0.247		0.466	659		1543	0.307		0.393	0.213		0.362	521		1198
	B	10		**		1, 14	0.445		0.584	0.398		0.764	1755		2530	0.461		0.592	0.420		0.781	1948		2586
2	A	15	killed	*/ 0.5ml	S/C	10.		0.580	0.583		0.833	0.762		1878	2523	0.543	0.584	0.586		0.842	0.768		1900	2543
	B	15		**/0.5ml		1, 22		0.639	0.625		0.974	0.850		2231	2815	0.610	0.675	0.634		1.060	0.869		2448	2878
3	A	15	Killed after live	*/ 0.5ml	Eye drops, S/C	°, 22	0.524	0.636	0.614	0.504	0.967	0.827	2776	2213	2738		0.654	0.623	0.529	1.010	0.846	3050	2321	2801
	B	15		**/0.5ml		°, 14, 33	0.584	0.797	0.675	0.584	1.351	0.956	3696	3197	3166		0.847	0.699	0.619	1.470	1.006	4138	3508	3331
Control		80	-ve				0.291	0.291	0.291	0.165	0.165	0.145	316	316	480	0.291	0.291	0.285	0.165	0.165	0.133	316	316	440

S/P value 0.350 or greater consider +ve (Mean –ve control OD was 0.222 and Mean +ve control OD was 0.696) for ND Log_{10} titre = 1.0* log (SP) + 3.52
S/P value > 0.139 is consider +ve (Mean –ve control OD was 0.148 and Mean +ve control OD was 0.894) for IB Log_{10} titre = 1.9426 x Log10 S/P + 4.0215
S/P value 0.2 or greater consider +ve (Mean –ve control OD was 0.231 and Mean +ve control OD was 0.650) for IBD Log_{10} titre = 1.1*Log (SP) + 3.361
V/D= Vaccination/Dose*= one vaccination **=Revacination °= priming with live vaccine day old IB, 7 days ND & 10 days IBD

Table 3. Immune response post vaccination with ND vaccines by using ELISA at different serum dilutions

group	subgrp	No. of birds	Vaccine				ELISA test												Challenge test
							Mean OD at 3 weeks Post						Mean OD at 4 weeks Post						Protection%
			Type	V/D	Route	Age/day	1:100	1:200	1:400	1:800	1:1600	1:3200	1:100	1:200	1:400	1:800	1:1600	1:3200	
1	A	10	Live	*	Eye drop	9	0.827	0.548	0.491	0.335	0.289	0.212	0.752	0.503	0.364	0.275	0.238	0.189	40%
	B	10		**		1, 14	0.966	0.686	0.598	0.483	0.375	0.311	0.987	0.640	0.584	0.510	0.464	0.348	80%
2	A	15	killed	*/ 0.5ml	S/C	10.	1.012	0.618	0.589	0.435	0.312	0.294	1.015	0.627	0.598	0.495	0.387	0.319	80%
	B	15		**/0.5ml		1, 22	1.130	0.793	0.689	0.575	0.492	0.362	1.172	0.816	0.674	0.596	0.500	0.380	100%
3	A	15	Killed afte live	*/ 0.5ml	Eye drops, S/C	7°, 22	1.218	0.856	0.678	0.560	0.473	0.354	1.275	0.845	0.683	0.565	0.489	0.368	100%
	B	15		**/0.5ml		7° 14, 33	1.359	0.906	0.788	0.593	0.515	0.421	1.413	0.953	0.798	0.628	0.587	0.456	100%
Control		80	-ve				0.186	0.136	0.119	0.109	0.103	0.092	0.199	0.143	0.124	0.105	0.099	0.096	-

V/D= Vaccination/Dose *=one vaccination **=Revacination °= Age of priming with live vaccine is considered -ve

88 (3.3±0.71) and 77(3.1±0.68) at 6 and 9weeks post vaccination, respectively *(17)*. While IBV live vaccines induced short live protection, the start of the decline being apparent 9 weeks after vaccination with vaccines based on highly attenuated strains, consequently, chickens may be re-vaccinated, with the same or another serotype, two or three weeks later *(18)*.

In case of vaccination with one dose of bivalent inactivated ND+IBD vaccine, at 3 weeks post vaccination, ELISA results showed positive values only in 5 serum dilutions. At 4 weeks post vaccination, all dilutions were positive for both ND and IBD. Revaccination with the same vaccine, all ELISA dilutions were not only positive at 3 weeks post vaccination, but also higher at 4 weeks post revaccination (Table 3, 5). Thirty days post vaccination with bivalent ND-IBD killed vaccine, IBD-ELISA revealed lower titre in chickens received single vaccination than chicken revaccinated with the same vaccine *(10)*.

Vaccination with trivalent (ND+IB+IBD) vaccine after priming with the live vaccines showed positive ELISA values at all serum dilutions for ND, IB and IBD at 3 and 4 weeks post vaccination. Chickens revaccinated with the same vaccine, recorded the highest mean OD value over all serum dilutions at 3 and 4 weeks post vaccination (Table 3 - 5). Broiler breeders, 30 weeks old, were vaccinated at 8th, 18th, 133rd day of age with live and killed vaccines against IBD, induced a high level of antibodies detectable by using indirect ELISA as a result of vaccination. The absorbance values (OD) were strongly positive, even higher than that of the other group of layer breeder birds (13 weeks of age) which were vaccinated thrice. Unvaccinated group showed negative OD values against IBDV, the higher OD values suggested that the higher dilutions of sera could be used and it was observed that almost all the samples that were found positive or negative with commercial ELISA kit also showed similar patterns with in-house ELISA, the pattern of increasing or decreasing OD values was quite similar *(20)*.

Screening sera by hemagglutination inhibition test (HI) at 3 and 4 weeks post vaccination with live bivalent ND+IB vaccine, the GMT range of antibodies against ND declined from 32 to 9.8, respectively, in vaccinated chickens at 9 days of age, while revaccination with the same vaccine at 14 days-old, the GMT range was higher (128-157.6) at 3 and 4 weeks post vaccination, respectively. On the other hand, vaccination with bivalent inactivated ND+IBD vaccine at 10 days of age, GMT range of antibodies increased from 90.5 to 128 at 3 and 4 weeks post vaccination, respectively. While GMT range was higher ranged (256-315.2), respectively, in chickens revaccinated with the same vaccine at 22 days-old.

In chickens vaccinated with inactivated trivalent ND+IB+IBD vaccine at 22 days of age after priming with live vaccine the GMT range of ND antibodies was increased from 274.4 to 415.9 at 3 and 4 weeks post vaccination, respectively, while in chickens revaccinated with the same vaccine at 33 days of age higher GMT were obtained (630.3 - 1448.2) at 3 and 4 weeks post vaccination, respectively (Table 6). These results agreed with previous study which showed that the HI GMT of vaccinated chickens with live ND vaccine was 50 and 48, while chicken vaccinated (s/c) with inactivated oil emulsion vaccine, the HI level was 71 and 168, On the other hand vaccinated groups with combined inactivated oil emulsion vaccine (s/c) plus live virus vaccines by oculo-nasal route, the HI GMT was 104 and 220 at 2 and 3 weeks post vaccination, respectively *(21)* and there was no significant difference between inactivated monovalent ND vaccine and polyvalent inactivated ND+IB+IBD, where the HI titre was 500 and 450, respectively, after one month of vaccination *(5)*. NDV-HI titers was significantly higher in chickens vaccinated via subcutaneous, lower thigh and thigh muscles with OILVAX at 1, 2, 4 and 8months post vaccination, 2632, 1597, 997, 1280 and 534 respectively, in comparison with chickens inoculated via shoulder and breast muscles route (1391, 676.5, 588.9, 586.9 and 357) and (1736.5, 931.6, 718.4, 798.9 and 361 respectively) *(22)*.

Table 4. Immune response post vaccination with IB vaccines by using ELISA at different serum dilutions

Group	subgrp	No. of birds	Vaccine				ELISA test											
							Mean OD at 3 weeks Post						Mean OD at 4 weeks Post					
			Type	V/D	Route	Age/day	1:100	1:200	1:400	1:800	1:1600	1:3200	1:100	1:200	1:400	1:800	1:1600	1:3200
1	A	10	Live	*	Eye drop	9	0.726	0.495	0.371	0.325	0.279	0.212	0.689	0.394	0.328	0.285	0.230	0.198
	B	10		**		1, 14	0.866	0.597	0.498	0.424	0.364	0.321	0.958	0.660	0.534	0.450	0.383	0.332
3	A	15	Killed after live	*/ 0.5ml	Eye drops, S/C	1°, 22	0.998	0.681	0.589	0.453	0.376	0.344	1.008	0.705	0.610	0.506	0.473	0.369
	B	15		**/0.5ml		1°, 14, 33	1.214	0.786	0.694	0.588	0.463	0.395	1.292	0.809	0.714	0.611	0.523	0.420
Control		50	-ve				0.189	0.178	0.164	0.135	0.128	0.106	0.196	0.187	0.165	0.138	0.120	0.099

V/D= Vaccination/Dose *=one vaccination **=Revacination

is considered –ve

Table 5. Immune response post vaccination with IBD vaccines by using ELISA at different serum dilutions

Group	subgrp	No. of birds	Vaccine				ELISA test												Challenge test
							Mean OD at 3 weeks Post						Mean OD at 4 weeks Post						Protection%
			Type	Dose	Route	Age/day	1:100	1:200	1:400	1:800	1:1600	1:3200	1:100	1:200	1:400	1:800	1:1600	1:3200	
2	A	15	killed	One/ 0.5ml	S/C	10	0.955	0.768	0.591	0.465	0.322	0.287	0.852	0.785	0.674	0.573	0.460	0.352	100%
	B	15		Two/0.5ml		1, 22	1.080	0.873	0.735	0.620	0.547	0.429	1.117	0.899	0.785	0.669	0.576	0.442	100%
3	A	15	Killed after live	One/ 0.5ml	Eye drops, S/C	10°, 22	1.146	0.958	0.791	0.634	0.538	0.410	1.199	0.964	0.824	0.653	0.549	0.438	100%
	B	15		Two/0.5ml		10°, 14, 33	1.291	0.998	0.822	0.735	0.612	0.549	1.315	0.986	0.868	0.756	0.637	0.564	100%
Control		80	-ve				0.198	0.152	0.146	0.134	0.115	0.107	0.185	0.162	0.150	0.141	0.123	0.111	-

°= Age of priming with live vaccine Cut off value at Optical Density (OD) 0.3 consider +ve and less is –ve

Table 6. Immune response post vaccination with ND by using HI

Group	subgroup	No. of birds	Vaccine				Hemagglutination Inhibition test				Challenge test 3weeks post vaccination			
							Post 3 weeks		Post 4 weeks					
			Type	V/D	Route	Age	Mean HI	GMT range	Mean HI	GMT range	diseased	deaths	survival	Protection%
1	A	10	Live	*	Eye drop	9d.o	5	32	3.3	9.8	2	1	2/5	40%
	B	10		**		1, 14d.o	7	128	7.3	157.6	1	-	4/5	80%
2	A	15	killed	*/ 0.5ml	S/C	10d.o.	6.5	90.5	7	128	1	-	4/5	80%
	B	15		**/0.5ml		1, 22d.o	8	256	8.3	315.2	-	-	5/5	100%
3	A	15	Killed after live	*/ 0.5ml	Eye drops, S/C	7°, 22d.o	8.1	274.4	8.7	415.9	-	-	5/5	100%
	B	15		**/0.5ml		7°, 14, 33d.o	9.3	630.3	10.5	1448.2	-	-	5/5	100%
Control		80	-ve				1.5	2.8	1.5	2.8	-	30	-	-

V/D= Vaccination/Dose *= one vaccination **= Revacination

Intramuscular inoculation with velogenic ND virus in a dose of $1ml x 10^6$ EID_{50}, showed 40% protection three weeks post vaccination with live bivalent ND and IB vaccine, while revaccinated chickens with the same vaccine showed 80% protection. On the other hand chickens vaccinated with inactivated bivalent vaccine ND+IBD at 10days of age, showed 80% protection. While chickens revaccinated with the same vaccine at 22 days of age showed 100% protection. Moreover vaccination at 22 days old and revaccinated chickens at 33 days old with trivalent inactivated vaccine ND+IB+IBD showed 100% protection (Table 3, 6). Similar results were obtained by performing potency tests for 27 different inactivated ND vaccines and recorded that GMT HI of at least 1:16 had 100% estimated protection and 89-100% actual protection while GMT HI titre below 1:16 had an estimated protection of ≥60% and their actual protection values were between 72 and 100% *(23)*.

Oral infection with IBD virus suspension ($1ml x 10^4$ EID_{50}) 3 weeks post vaccination, showed 100% protection in all chicken groups either vaccinated with killed bivalent ND and IBD vaccine or killed trivalent ND+IB+IBD vaccine. While chickens of the control group showed no protection post challenge and the bursa index, bursa weight and the body weight were 2.4, 2.75gm and 1145 gm respectively (Table 5). All IBD vaccines were equally capable of protecting chickens against challenge at 35 days with smaller bursa and moderate microscopic bursal lesions *(24)*.

Single dilution ELISA (1:500) confirmed different dilutions ELISA (1:100 to 1:3200) results for ND, IB and IBD and correlated with HI results and challenge test. Birds receiving one dose of live HB1 vaccine exhibit positive ELISA 688 and HI (6.2) titres to NDV, while birds vaccinated subcutaneously with single dose of inactivated vaccine displayed positive NDV ELISA 4045 and HI (9) titres, 2 weeks post vaccination, the antibody response to NDV was significantly higher in the groups receiving inactivated vaccine than live vaccine on all days tested *(19)* and it has been recorded that the efficiency of the in house developed ELISA was compared with commercially ELISA kit and results indicated that they were equally sensitive and specific in detection of antibodies against IBD *(20)*.

It is concluded that immunization of broilers with trivalent inactivated vaccines after priming with live vaccine is the most preferable vaccination program in controlling Newcastle disease, Infectious Bronchitis and Infectious Bursal diseases.

REFERENCES

1.Babiuk LA, Gomis S and Hecker R. (2003): Molecular approaches to Disease control. Poultry Science; 82:870-875.

2.Alexander DJ (1997): Newcastle disease and other avian Paramyxoviridae infections. In: Canlek, B.W., Barnes, H.J., Beard, C.W.,McDougald, L.R.,Saif, Y.M. (Eds.), Diseases of Poultry, 10th edn., Iowa State University Press, Ames IA, pp. 541-569.

3.Muhammad Wasif Malik, Najma ayub, Irfan Zia Qureshi (2006): Passive immunization using purified IgYs against infectious bursal disease of chickens in Pakistan. J. Vet. Sci., 7(1), 43-46.

4.Hofstad M (1984): Avian infectious bronchitis. In: Diseases of Poultry, Iowa State University Press, pp. 429-443.

5.Petko Tzvetkov, Krassimira Genova, Nezabravka Djambazova (2000): Preparation of trivalent inactivated vaccine against Newcastle disease, infectious bursal disease and infectious bronchitis in chickens. Exp. Pathol.& Parasito; Apr:31-34.

6.Office International des Epizooties (OIE 2004): Manual of standards for Diagnostic tests and Vaccines 2004.

7.Marqurdt WW, Johnson RB, Odenwald WF and Schlotthoken BA (1980): An indirect enzyme-linked immunosorbent assay (ELISA) for measuring antibodies in chickens infected with infectious bursal disease virus. Avian Dis., 24:375-385.

8.De Wit JJ, Mekkes DR, Koch G and Westenbrink (1998): Detection of specific IgM antibodies to infectious bronchitis virus by an antibody-capture ELISA. Avian Pathol. 27:155-160.

9.Snyder DB, Marquardt WW, Mallinson ET, Savage PK and Allen DC (1984): Rapid Serological Profilingby Enzyme-Linked Immunosorbent Assay. III. Simultaneous Measurements of Antibody Titers to Infectious Bronchitis, Infectious Bursal Disease and Newcastle Disease viruses in a Single Serum Dilution. Avian Dis., 28 (1): 12-24.

10.Giambrone JJ and Clay RP (1986) (a): Vaccination of day-old broiler chicks against Newcastle disease and infectious bursal disease using commercial live and/or inactivated vaccines. Avian Dis., 30(3):557-61.

11.Keck LD, Skeeles JK and McNew RS (1993): Antibody Detection in Matched Chicken Sera and Egg-Yolk Samples by Commercial Enzyme-Linked Immunosorbent Assay Kits for Newcastle Disease Virus, Infectious Bronchitis Virus, Infectious Bursal Disease Virus and Avian Reovirus. Avian Dis., 37:825-828.

12.Mutalib Ahmed and Boyle Carolyn R. (1994): Influence of Site of Inoculation of Inactivated Vaccines on the Immune Response of chickens. Avian Dis., 38:857-860.

13.Maas RA, Oei HL, Venema-Kemper S, Koch G and Bongers J (1999): Dose-Response Effects of Inactivated Newcastle Disease Vaccines: Influence of Serologic Assay, Time After Vaccination, and Type of Chickens. Avian Dis., 43:670-677.

14.Ming Kun Hsieh, Ching Ching Wu and Tsang Long Lin (2007): Primming with DNA vaccine and boosting with killed vaccine conferring protection of chickens against infectious bursal disease. Vaccine 25:5417-5427.

15.Mockett APA and Darbyshire (1981): Comparative studies with an Enzyme Linked Immunosorbent Assay (ELISA) for antibodies to avian Infectious Bronchitis virus. Avian Pathol. 10:1-10.

16.Ladman BS, Pope CR, Ziegler AF, Swieczkowski T, Callahan CJ, Davison S and Gelb JJr (2002): Protection of chickens after live and inactivated virus vaccination against challenge with nephropathogenic infectious bronchitis virus PA/Wolgemuth / 98. Avian Dis., 46 (4): 938-44.

17.Illango J, Olaho-Mukani W, Mukiibi-Muka G, Abila PP and Etoori A (2005): Immunogenicity of a Locally Produced Newcastle Disease I-2 Thermostable Vaccine in Chickens in Uganda. Tropical animal health and production, 37:25-31.

18.Gavanagh D (2003): Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. Avian Pathol. 32(6):567-82.

19.Kapczynski Darrell R and King Daniel J (2005): Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. Vaccine 23:3424-3433.

20.Amin F, Ahmad MD, Muhammad K and Chaudhary ZI (2008): Standardization of an Indirect Enzyme Linked Immuno Sorbent Assay for Measuring Antibodies of Infectious Bursal Disease Virus. J. Vet. Anim. Sci. Vol. 1:18-23.

21.Raphael Folitse, Halvorson DA and Sivanandan V (1998): Efficacy of Combined Killed-In-Oil Emulsion and Live Newcastle Disease Vaccines in Chickens. Avian Dis., 42:173-178.

22.Kazuhiro Deguchi, Takashi Honda, Kazuo Matsuo, Hideo Fujikawa, Toshinori Iwamoto and Yoshihiro Sakanoue (1998): Influence of Inoculation Site of Combined Oil-Adjuvanted Vaccine on the Antibody

Response in Chickens. J. Vet. Med. Sci.; 60(7): 831-835.

23. ***Horvath E, Czifra G, Nagy E, Engstrom B and Merza M (1999):*** Potency test of inactivated Newcastle disease vaccines by monoclonal antibody blocking ELISA. Vaccine 17:2969-2973.

24. ***Giambrone JJ and Clay RP (1986) (b):*** Evaluation of the immunogenicity, stability, pathogenicity, and immunodepressive potential of four commercial live infectious bursal disease vaccines. Poultry Science; 65(7):1287-90.

الملخص العربى

تقييم الأستجابة المناعية للدجاج بعد التحصين ببعض اللقاحات الفيروسية

أحمد محمد الصادق حجازى* ، لماح كامل عبد السميع**

*أستاذ ورئيس قسم طب الطيور والأرانب بكلية الطب البيطرى - جامعة الزقازيق

**طبيب بيطرى ومدير المستشفى البيطرى المتنقل بكلية الطب البيطرى - جامعة الزقازيق

فى هذه الدراسة تم تقييم الأستجابة المناعية لعدد مائه وستون كتكوت بعد التحصين بلقاحات ثنائية و ثلاثية ضد مرض النيوكاسل، الألتهاب الشعبى المعدى ومرض الجامبورو باجراء إختبارات الآليزا والتلازن الدموى والتحدى. دلت نتائج إختبارات الأليزا أحادي التخفيف (١:٥٠٠) و الأليزا متعددة التخفيف (١:١٠٠-١:٣٢٠٠) وأختبار التلازن الدموى ان التحصين باللقاح الحى الثنائى (نيوكاسل و الألتهاب الشعبى المعدى) عند عمر تسعة أيام لمرة واحدة ادى الى أنخفاض الأجسام المناعية بعد ٤ أسابيع من التحصين بينما أرتفعت فى الدجاج المحصن بجرعتين وكذلك التحصين باللقاح الميت الثنائى (نيوكاسل و الجامبورو) حيث كانت الأجسام المناعية فى الدجاج الذى أعيد تحصينه أعلى من المحصن مرة واحدة. وقد سجلت المجموعات المحصنه بلقاح حى ثم حصنت باللقاح الميت الثلاثى (نيوكاسل والألتهاب الشعبى المعدى والجامبورو) أعلى مستوى للأجسام المناعية. مما سبق تأكد توافق الأختبارات السيرولوجية مع بعضها البعض ومع نتائج أختبار التحدى حيث كانت نسبة الحماية من العدوى ١٠٠%.