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International Journal of Current Research
Vol. 7, Issue, 01, pp.11608-11617, January, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EVALUATION THE BREADTH OF PROTECTION OF SOME AVAILABLE COMMERCIAL LIVE IB VACCINES AGAINST AN EGYPTIAN VARIANT (EG/1212B) OF IBV

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ARTICLE INFO

Article History:
Received 22nd October, 2014
Received in revised form
14th November, 2014
Accepted 19th December, 2014
Published online 23rd January, 2015

Key words:

Infectious bronchitis,
Vaccine,
Ciliary protection,
Challenge,
Nephritis.

ABSTRACT

The present study was conducted to estimate the breadth of the protection against the characterized Egyptian (EG/1212B) strain of IBV and evaluation of different immunization programs using some available commercial live vaccines in Egypt as following; group (1) vaccinated by (IB Primer and Ma 5), group (2) vaccinated by (IB Primer and 4/91), group (3) vaccinated by (Ma5 and H120), group (4) are non-vaccinated challenged and group (5) are non-vaccinated non-challenged. At 26th days, the chickens in groups (1,2,3 & 4) were individually challenged with (10^5 EID_{50}/bird) IBV (EG/1212B) strain. The results indicated that group (1) recorded the highest degree of ciliary protection by 82%, followed by the second group by 56%, then the third group by 32% in relation to control non-vaccinated non-challenged group (5). Groups No. (1, 2, 3 and 4) secreted the challenge virus at 5days PC at different rates and group (1) had the lowest rate of virus secretion. Group (2) recorded the highest serological response to vaccination at 26th days (day of challenge) with 80% positive random samples. Experimentally challenged chicks showed varying degrees of coughing, sneezing, tracheal rales, head shaking, depression and watery feces. No mortalities were recorded in all five groups. The main common lesions were swollen and congestion of kidneys together with tubules and ureters distineted by urate. There were sticky clear mucoid secretion in orophraynx and trachea of groups (2, 3 and 4). Histopathological finding PC, exhibited tracheal lesions of varying degree of deciliatin, thickning in lining epithelium accompanied with inflammatory cells infiltration and vacuolation of goblet cells. In addition to renal lesions with varying degree of focal interstitial nephritis and hydrobic degeneration. The impact of the three immunization programs on the rate of body weights gain of vaccinated birds at 26th days (day of challenge) and at 7 days PC had recorded.

INTRODUCTION

Infectious bronchitis (IB) disease is an acute, highly contagious and infectious disease of poultry in worldwide, possess a major threat to the poultry industry and was first reported in North Dakota, USA, as a novel respiratory disease by Schalk and Hawn (1931). The disease is characterized by respiratory signs including (sneezing, cough, tracheal rales, gasping and nasal discharge), reduction the growth rate of broilers, nephropathogenic strains causing acute nephritis, urolithiasis and may be associated by high mortality (Linda, 2006). The transmission of IBV is mainly via the respiratory tract from infected chickens. Infection occurs via inhalation of droplets containing the air born virus, which may travel several kilometers. In Egypt, IB was first described by Ahmed (1954), subsequently several reports (Abdel Moneim *et al.,* 2002; Sultan *et al.,* 2004; Lebdah *et al.,* 2004; Sedeik, 2005 and 2010) emphasized the prevalence of the disease. The Egyptian variants which were closely related to the Israeli variant strain were isolated from different poultry farms (Abdel-Moneim *et al.,* 2002; Sedeik, 2010).

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Live attenuated and inactivated vaccines have been available to control IB for many decades. The most commonly used vaccine strains are representatives of the Massachusetts and Connecticut antigenic groups, and they are reasonably effective in controlling clinical disease and production losses associated with IBV infection (Winterfield *et al.,* 1976; Cavanagh, 2003). However, the continuous emergence of new IBV variants as a consequence of mutation and recombination of the virus genome remains a problem for both the poultry industry and vaccine manufacturers (Nix *et al.,* 2000).

Extensive clinical experience and laboratory studies have shown that vaccination with two or more different live attenuated IBV vaccines confers a broad protection against many important heterologous serotypes (Cook *et al.,* 1999 and 2001, Worthington *et al.,* 2004). This has led to the ‘‘protectotype’’ concept, according to which significant cross-protection can be obtained by using strains that are dominant antigenically. The aim of study is to evaluate breadth of protection provided by some commercially available live attenuated IBV vaccines in Egypt against challenge with the characterized IBV(EG/1212B) variant strain.

MATERIALS AND METHODS

SPF chicks

A total of 100 SPF chicks hatched from SPF eggs (obtained from Specific pathogen free governmental farm, Kom Oshim, Fayoum, Egypt) were used for experimental infection and challenge tests. They were raised in isolated pens with negative pressure and fed ad-libidum with their recommended commercial broiler ration.

IBV vaccines used in vaccination-challenge experiments

Four different commercially available vaccines were used; they were administered at the manufacturer's recommended dose by the oculonasal (O.N.) route.

Table 1. IBV vaccines used in vaccination-challenge experiments

Vaccine	Lot Number:	Production Date:	Expiry Date:	Company
Poulvac® IB Primer	1300876D3	1-2013	1-2015	pfizer
Nobilis® IB Ma5	A157A1J02	11-2013	11-2015	Intervet
Nobilis® IB 4/91	A125A1J01	9-2013	9-2014	Intervet
Nobilis® IB H120	12638F01	11-2012	11-2014	Intervet

IBV challenge strains

The challenge strain (IBV/EG/1212B) had been identified in Reference Laboratory for Quaulity control on poultry production (RLQP) as variant II strain and it was related to ***IS/1494/06.***

Gene accession no. (JQ839287).

The virus used in the challenge in the form of infectious allantoic fluid at the level of fifth–passage.

Determination of embryo infective dose-$_{50}$ (EID_{50}) of the challenge strain used for challenge tests

This was carried out after (Villegas and purchase, 1989). Allantoic fluid of the challenge strain (IBV/EG/1212 B), was ten-fold diluted (10^{-4} to 10^{-8}) in PBS and inoculated in 9-day-old SPF embryonated eggs via the allantoic sac (4 eggs/ dilution and 0.lml /egg). The eggs were incubated at 37^{o}C and candled daily for mortality up to 5 days post inoculation (PI). The dead embryo within 1st 24 hrs were discarded as non specific mortality. Embryos which died later and survivors were opened 5 days PI and the characteristic embryonic change of IBV infection were recorded. The EID_{50} was calculated according Reed and Muench (1938), using the embryo gross pathological changes (dwarfing) as criteria for IBV infection.

Experimental design

- Five groups of one-day old SPF(specific pathogen free) chicks were used (20 chicks/group).
- The chichs were housed in negative-pressure isolators. Bioflex B50 (Bell labs, England).
- Groups 1, 2, and 3 received one full dose as recommended by the manufacturer of the attenuated vaccine strain of Poulvac IB Primer, Poulvac IB Primer, and Nobilis IB MA5 respectively at the first day of age via the ocular-nasal route (50 ul/chick).
- Groups 4 and 5 remained unvaccinated till 26 days of age.
- At 12th day of age the first three grougs (1, 2, 3) were received a booster dose the attenuated vaccine strain (Nobilis IB Ma5, Nobilis IB 4/91, Nobilis IB H120) respectively.
- Ten Serum samples were collected from each group at 26th day of age (2 weeks from second vaccination) before challenge to determine IBV antibody titers by ELISA.
- Average body weights gain for each group were estimated at 26th day before virus challenge and at 7 days post challenge.
- At 26th day of age groups 1, 2, 3and 4were experimentally infected via the intra-ocular and intranasal routes with the (EG/1212B) strain of IBV (10^{5} EID_{50}/bird) kindly provided by RLQP Egypt (100 ul/ bird)
- A negative control group (Group 5) was remained as non vaccinated-non challenged and maintained under the same conditions.
- Each group was inspected for 10 days post challenge for the onset of clinical signs, degree of respiratory signs , morbidity, mortality rate andcourse of the disease .
- Orophryngeal swabs and kidney samples were collected from each group at 5 days PC. And immediately frozen and kept at -70 C until transferred to PCR for detection of challenge virus shedding.
- Five chickens from each group sacrificed at 5 days PCby inducing air embolism through inoculating air inside the heart. Tracheal samples were collected from each group and technically processed for ciliary kinetic analysis (ciliostasis analysis) and histopathological examination.
- Kidney samples collected at 10 days PC from each groups for histopathological examination.

Scoring indexes for clinical and lesions: Were recorded according to (**Avellaneda *et al*., 1994; Wang and Huang, 2000)** as follows:

a) Clinical signs score system of infected chickens

Score 0 = No clinical signs;
Score 1 = lacrimation, slight shaking of head, watery feces;
Score 2 = lacrimation, presence of nasal exudate, depression, watery feces;
Score 3 = strong (lacrimation, presence of nasal exudate, depression, severe watery feces).

Gross lesions scores of kidney

Score 0 = no lesions;
Score 1 = swelling, urate visible only under stereomicroscopy;
Score 2 = swelling with visible urate;
Score 3 = swelling with large amount of urate deposit in kidney.

Table 2. The experimental design

Group No.	Day 1	Day 12	Day 26	Day 31	Day 36
1	Vaccinated by IB primer	Vaccinated by IB MA5	-Ten bloods samples	-Oropharyngeal swabs for RCR	kidney samples for
2	Vaccinated by IB primer	Vaccinated by IB 4/91	- average body weight gain	-tracheal samples of 5 birds for	Histopathological
3	Vaccinated by IB MA5	Vaccinated by IB H120	- Challenged by isolate	TOC and Histopathological	examination
4	Non Vaccinated	Non Vaccinated	(IBV/EG/1212B)	examination	
5	Non Vaccinated	Non Vaccinated	-Ten bloods samples		
			-non Challenged		

Ciliostasis test

Protection of the respiratory tract provided by different commercially available live-attenuated IBV vaccines against challenge with IBV (EG/1212B) strain. Five chicks from each group were humanely killed. The tracheas were carefully removed and examined for ciliary activity as following:
From each bird five 1 mm to 2mm sections were prepared from trachea (two sections from upper part of trachea – one from middle - two sections from lower). Each of 5 explants prepared from one trachea in Petridish containing minimum essential medidium (MEM) was examined by low-power microscopy and ciliary activity scored as follows: (4) all cilia beating; (3) 75% beating; (2) 50% beating; (1) 25% beating; and (0), none beating (100% ciliostasis). This gave a maximum possible score of ciliary activity for a trachea of 20. The higher the score, the higher the level of protection provided by that vaccination program.

An individual chick was recorded as protected against challenge if the ciliary beating scores more than 10 or ciliostasis score for that trachea was less than 10.

Scoring the ciliary activity according to (Cook *et al.,* 1999 modified)

4: 100% ciliarybeating (no ciliostasis).
3: 75% ciliary beating (25% ciliostasis).
2: 50% ciliary beating (50% ciliostasis).
1: 25% ciliary beating (75% ciliostasis).
0: 0% ciliary beating (100% ciliostasis).

For each group a protection score was calculated according to the modification of formula proposed by (**Cook *et al.,* 1999***)* as follow:-

$$\left[\frac{\text{Mean ciliary beating score for vaccinated challenged group}}{\text{Mean ciliary beating score for corresponding non vaccinated non challenged group}}\right]\times 100$$

Histopathological examination

Specimens from tracheas and kidneys were collected from experimentally infected birds sacrificed at the 5th and 10th day PI and immediately preserved in 10% neutral buffered formalin then processed through paraffin embedding technique. Sections of 5-10 um in thickness were prepared and stained with haematoxylin and eosin (H&E) stain (Culling, 1983).

Enzyme-Linked Immunosorbent Assay (Indirect ELISA)

IBV antibody test kits were supplied by Synbiotics Corporation, U.S. (Cat.No.13005/2) ELISA reader (spectrophotometer with 405-410nm filter). *Asys Expert Plus UV* Microplate Reader - Biochrom, Australia and ELISA program. ELISA test was carried out on serum samples collected from experimentally vaccinated birds for antibody detection. The test was performed according to the directions of the kit producer company.

RESULTS

Results of virus titration

The selected (IBV/EG/1212B) strains of study was titrated in embryonated SPF eggs to determine its EID_{50} before being used as challenge IBV. The results are shown in Table (3). The estimated EID_{50} was 10^6. With Ct- value of (15.65).

Results of spectrum of cilaiary beating score (ciliary protection)

The definitive presence or absence of ciliary movement in each explant enabled a quantal estimate to be made of the response to the challenge strain of virus. And the ciliary protection among the vaccinated groups ranged from 32 % to 82%. According to modification of formula proposed by (Cook *et al.,* 1999).

Table 3. Estimation of Embryo Infective Dose 50% of (IBV/EG/1212B) using Reed and Muench

Virus dilution inoculated	Embryos.		Accumulated number		Proportion dwarfed/Total	Percent Dwarfed
	No. of dwarfed embryos	No. of normal embryos	dwarfed embryos	normal embryos		
10^{-4}	4	0	13	0	4/13	30.7%
10^{-5}	3	1	9	1	3/9	33.3%
10^{-6}	3	1	6	2	3/6	50%
10^{-7}	2	2	3	4	2/3	67%
10^{-8}	1	3	1	7	1/1	100%

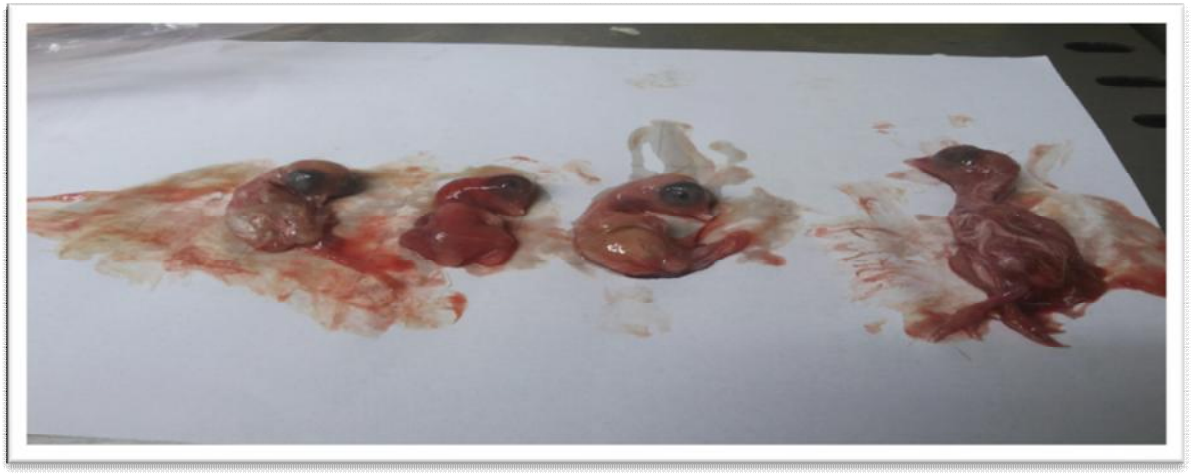


Figure 1. Curling and dwarfing of embryos induced by variant (IBV/EG/1212B) in comparison to control non infected embryo

Table 4. Percentage of ciliary beating score (ciliary protection) 5 days PC of SPF chickens by Egyptian IBV (EG/1212B) variantstrainvaccinated with combined different vaccination program

Group no	*IB vaccines*		*Ciliary beating score*					% of ciliary beating score /bird	% of ciliary beating score/ Group
	At one day old	At 12 day old	2 tracheal ring from upper part of trachea		1 tracheal ring from middle part of trachea	2 tracheal ring from lower part of trachea			
			4	1	2	3	3	65%	
1	IB Primer		3	3	4	4	4	90%	
		IB MA5	4	2	3	4	3	80%	77%
			4	1	4	4	3	80%	
			3	2	2	3	3	70%	
			0	0	1	1	3	25%	
2	IB Primer	IB 4/91	2	2	3	3	2	60%	
			4	3	2	3	2	70%	53%
			3	0	1	2	2	40%	
			3	4	2	2	3	70%	
			2	1	0	0	0	15%	
3	IB MA5	IB H120	2	3	2	1	2	50%	
			1	3	1	1	0	30%	30%
			1	2	1	1	0	25%	
			0	0	0	0	0	0%	
4	Non vaccinated		0	0	0	0	0	0%	
	challenged		0	0	1	0	0	5%	2%
	Control +ve		0	0	0	0	0	0%	
			0	0	0	1	0	5%	
			4	4	4	4	4	1OO%	
	Non vaccinated non		4	3	4	4	4	95%	
5	challenged Control –ve		4	4	3	3	4	90%	94%
			4	4	3	4	4	95%	
			4	3	4	4	3	90%	

Table 5. Results of IBV antibody response of ten random serum samples by the commercial ELISA kit at 26 days of age (2 weeks from second vaccination) of SPF chickens

Groups No.	1	2	3	4	5
treatment	IB Primer at 1 day IB MA5 at 12 day	IB Primer at 1 day IB 4/91 at 12 day	IB MA5 at 1 day IB H120 at 12 day	Non vaccinated challenged (Control +ve)	Non vaccinated non challenged (Control -ve)
Titer	0	440	0	0	0
	330	0	894	0	0
	0	259	0	0	0
	190	190	0	0	0
	0	2102	479	0	0
	595	5108	561	0	0
	350	0	0	0	0
	0	813	0	0	0
	275	871694	150	0	0
			0	0	0
Mean titer	174	1048	208	0	0
Positive no	5	8	4	0	0
Positive %	50%	80%	40%	0 %	0 %

Results of IBV antibody monitoring ELISA test

The antibody response to IBV vaccine was estimated at 26th days of age and the mean IBV antibody titer are 174, 1048 and 208 for group 1,2 and 3, respectively (Table 5).

Result of real RT-PCR

All vaccinated- challenged groups and control positive group secreted the challenge virus with different rate at 5 days PC from pooled oropharyngeal and kidney swabs (Table 6), and expressed as positive amplification curve using Step-One applied biosystem (Figure 2).

Result of average body weights gain

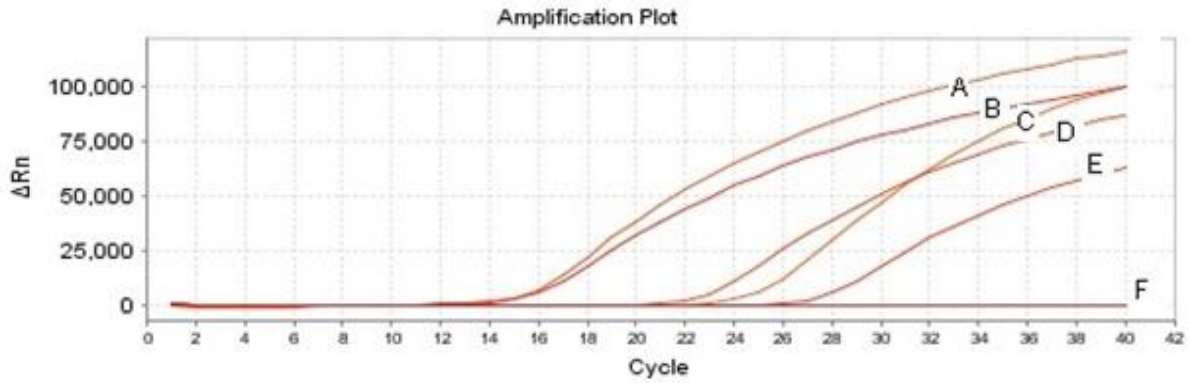
The average body weights gain of vaccinated chickens at 26th before IBV challenge were not significantly different but less in weight than non -vaccinated groups. At 7 days post challenge, the body weights gain of chickens in groups (1,2 & 3) which had received the vaccine was better than that of group (4), the positive challenged control. The body weights gain affected in groups (1&2) more than group (3).

Results of clinical signs and gross pathological lesions

For each group, the scores were pooled and the final score were the average of the pooled scores, the clinical scores were scored in Table (8).

Table 6. Ct value of real RT-PCR for IBV 5 days PC from pooled oropharyngeal and kidney swabs of experimentally challenged SPF chicken groups

Sample	Target	Quantity (Mean)	Quantity (StdDev)	C (Mean)	C (StdDev)
Group (1)	Target 1			15.90	
Group (2)	Target 1			16.65	
Group (3)	Target 1			23.00	
Group (4)	Target 1			27.89	
Group (5)	Target 1				
Standard	Target 1			24.81	



A= Group (4) B= Standard C= Group (3) D= Group (2) E= Group (1) F= Group (5)

Figure 2. Amplification curve of oropharyngeal and kidney swabs 5 days PC using Step-One applied bio system

Table 7. Average body weights gain of SPF chickens vaccinated groups and non vaccinated control groups at 26th days before IBV challenge and at 7 days post challenge

Groups	Average body weights gain	
	At 26th day (before challenge)	At 7th days post challenge
Group (1): IB primer + Ma5	225gm	290 gm
Group (2): IB primer + 4/91	220gm	288 gm
Group (3): MA5+ H120	228gm	292 gm
(4)Non vaccinated control group	230gm	284 gm
(5)Non vaccinated control group	232gm	298gm

Figure 3. Congestion of kidney of experimentally infected 31-day old SPF with isolate (EG/1212B) in group (4)

Table 8. Clinical and kidney lesion scores of SPF chicken groups challenged at 26 days old by (IBV/EG/1212B) strain

Group No.	Treatment	Observation within 10 days post challenge		Clinical score	kidney lesion scores in 5 sacrificed bird 10 day PC
		Morbidity rate	Mortality		
1	IB Primer at 1 day IB MA5 at 12 day	10/20 (50%)	0	0.85	0.6
2	IB Primer at 1 day IB 4/91 at 12 day	11/20 (55%)	0	1.0	0.4
3	IB MA5 at 1 day IB H120 at 12 day	14/20 (70%)	0	1..35	1
4	Non vaccinated challenged (Control +ve)	18/20 (90%)	0	2.0	1.6
5	Non vaccinated non challenged (Control -ve)	0/20 (0%)	0	0	0

PC= post challenged No. = Number

Experimentally challenged chickens showed varying degrees of coughing, sneezing, tracheal rales, head shaking, depression and watery feces. No mortalities were recorded in all five groups. The main common lesions were swollen and congestionof kidneys together with tubules and ureters distineted by urate. There were sticky clear mucoid secretion in orophraynx and trachea of groups (2, 3 and 4).

Results of histopathological examination

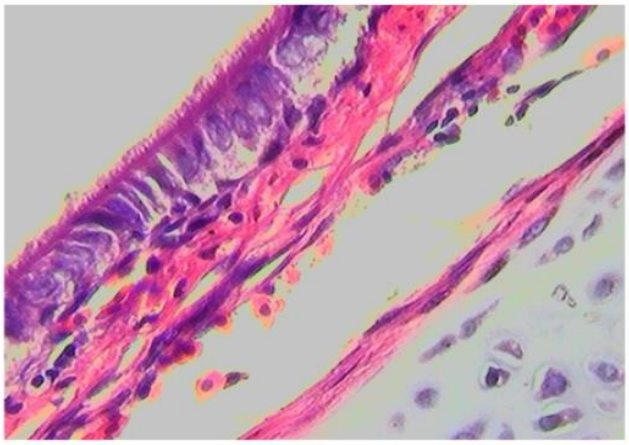


Figure 4. Trachea of 31-day-old SPF chicks non vaccinated non challenged (group 5). Note the intact cilia and the normal squamous epithelial cell

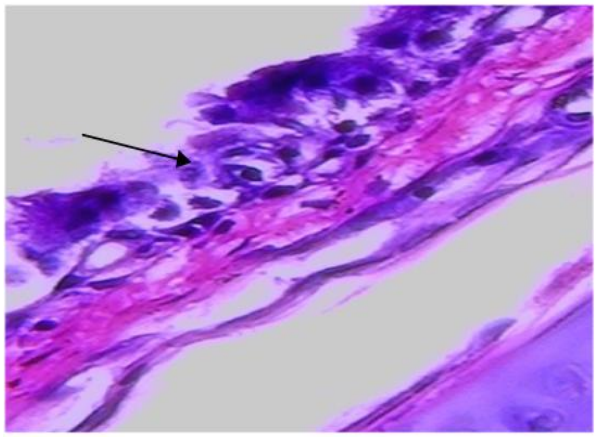


Figure 5. Trachea of 31-day-old SPF chicks non vaccinated challenged by Ref. strain (EG/1212B) (group 4). Note the cuboidalization of epithelial cells, loss of cilia (arrow), and inflammation and edema of the tracheal mucosa

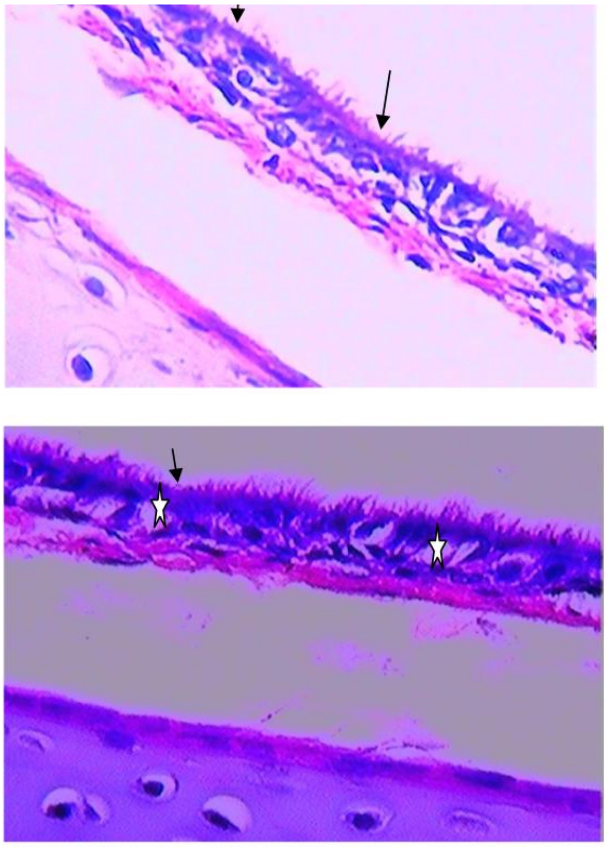


Figure 6. Group (1) Trachea of 31-day-old SPF chicks vaccinated (IB Primer+Ma5) challenged by Ref. strain (EG/1212B). Note Partial deciliatin (arrow) mild hypertrophy in lining epithelium and mild vacuolation of goblet cells (Star)

DISCUSSION

IB was controlled primarily by using live attenuated virus vaccine (e.g., H120, Ma5, commercially available and registered in Egypt) as well as inactivated oil emulsion vaccine, but more than sixty serotypes of IBV have been reported from all over the world (Ignjatovic and Sapats, 2000). So, it is useful for implementation of control measure to determine which IBV serotype(s) have been circulating in region as, protection provided by vaccination with a vaccine of a given serotype, is directed mainly against homologous serotype and less against strains of other serotypes (Davelaar *et al*., 1984).

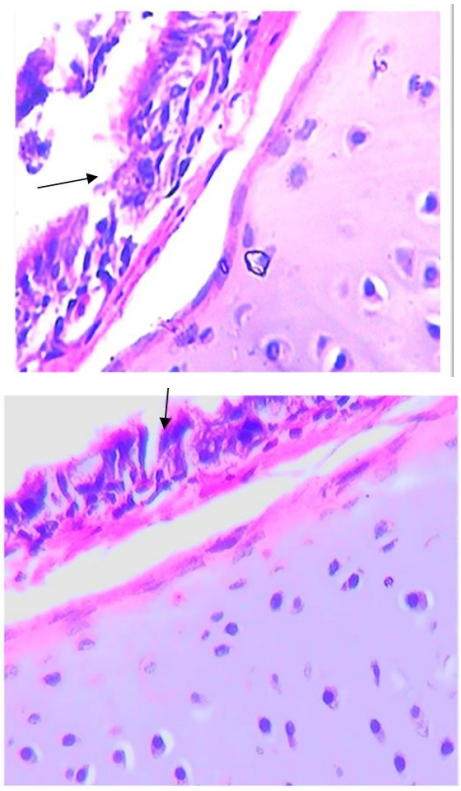


Figure 7. Group (2) Trachea of 31-day-old SPF chicks vaccinated (IB Primer+4/91) challenged by Ref. strain (EG/1212B). Note large areas of deciliatin and encryptation of Goblet cell (arrow), mild hypertrophy and moderate thickening in lining epithelium

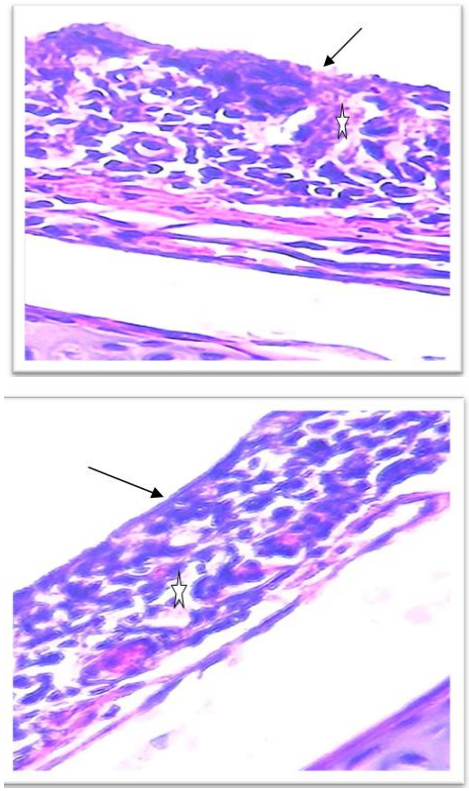


Figure 8. Group (3) Trachea of 31-day-old SPF chicks vaccinated (Ma5+H120) challenged by Ref. strain (EG/1212B). Note extensive deciliatin and sever hypertrophy and thickning in lining epithelium accompanied with inflammatory cells hemorrhages and vacuolation of goblet cells (Star)

In the present study IBV challenge strain (EG/1212B) had been identified in Reference Laboratory for Quality control on poultry production (RLQP) as variant II strain and it was related to IS/1494/06. It isused at dose of 100 ul $X10^5$ EID_{50} /bird to evaluate the breadth of protection of different vaccination programs using classic vaccine (Ma5 +H120), variant (IB Primer+ IB 4/91), and combined (IB Primer + Ma5). Live vaccines were administered by oculo-nasal route in order to ensure that each chick received the required dose of vaccine (Cook *et al.,* 1999). Also many authors have demonstrated IBV-specific IgA in the lachrymal fluid (Davelaar *et al.,* 1982*; Cook et al.,* 1992; Toro *et al.,* 1994) and its synthesis in the Harderian. In addition, the Harderian gland of chicken contains a large age-dependent population of plasma cell and is the source of immunoglobulins in the lachrymal fluid (Baba *et al.,* 1988). It playsan important role in development of vaccinal immunity since vaccines aregenerally given by spray or eye drop.

Generally, three main approaches to the assessment of protection have been (1) observation of clinical signs; and removal of trachea at 4 or 5 days after challenge followed by either (2) quantitative assessment of ciliary activity or (3) detection of live challenge virus, usually by inoculation of embryonated eggs (Cavanagh, 2003). The second and the third methods result in similar deductions being made as regards protection (Marquardt *et al*., 1982).

In the present study, the assessment of protection was depending on four approaches: (1) Assessment of ciliary activity using low power lens of light microscope. (Cook *et al.,* 1999), (2) Detection of the challenge virus using RRT-PCR (Meir *et al.,* 2004; Cook *et al.,* 2001), (3) Histopathological changes of both kidney and trachea (Cook *et al.,* 2001). (4) Observation of clinical signs, mortalities, and necropsy findings of both kidney and trachea (Mahgoub *et al.,* 2010), (5) Average body weights gain before challenge and at 7 days post challenge (Sasipreeyajan *et al*., 2012). Determination of level of antibody after vaccination by ELISA not for assessment of protection as it does not correlated with protection, but local antibody is believed to play role in of respiratory tract (Ignjatovic and Galli, 1994).

In the present study, Ciliostasis test (IB Primer + Ma5) show the highest level of ciliary protection about 82% then (IB Primer + IB 4/91) about 56% and (Ma5 +H120) about 32%, this results similar to results which recorded by (Hassanein, 2013 and Ali, 2014). The three vaccination programs failed to prevent the secretion of the challenge virus in trachea and kidney and were detected in the pooled samples with different rate, this results similar to results which recorded by (Hassanein, 2013). Vaccinated groups (1, 2 and 3) recorded varying degrees of coughing, sneezing, tracheal rales, head shaking, depression and watery feces, but less than that recorded in group 4 (non-vaccinated challenged). Also, number of bird affected in vaccinated groups (1, 2 and 3) less than number of bird affected in group 4 (non-vaccinated challenged), Table (8) this results similar to results which recorded by (Mahgoub *et al.,* 2010). No mortalities were recorded in all five groups because protection occur under the laboratory conditions and a lack of additional stressors such as

E. coli (Smith *et al.,* 1985) or avian Mycoplasma (Yoder *et al.,* 1977) infection. Cold stress may have also been a factor when the isolates were originally obtained. Also the most likely reason for mortality in the field is lack of sufficient vaccine distribution to all of the birds in the flock.

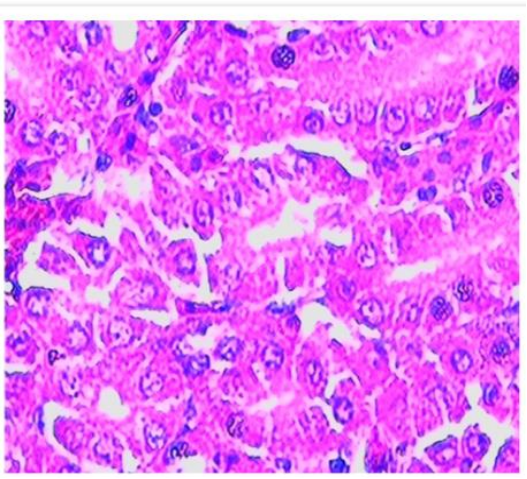


Figure 9. Normal histological renal structure of 36-day-old SPF chicks the non-vaccinated non challenged group (group 5) (H&EX40)

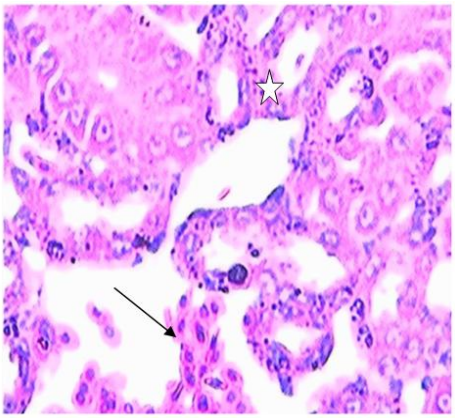


Figure10. Kidney of 36-day-old SPF chicks the non-vaccinated challenged group(group 4). Note vacuolar hydropic degeneration and hemorrhages (arrow) and peritubular lymphocytic infiltrations(Star). (H&EX40)

Group, 2 vaccinated with (IB Primer + IB 4/91) showed high level of circulating antibodies by using ELISA, but it does not correlated with protection, as local antibody is believed to play role in of respiratory tract (Ignjatovic and Galli, 1994). In our study , the average body weights gain of vaccinated chickens at 26th before IBV challenge were not significantly different but less in weight than non -vaccinated groups and this effect on weight may be due to the post vaccine reaction of live vaccines. At 7 days post challenge, the body weights gain of chickens in groups (1,2 & 3) which had received the vaccine was better than that of group (4), the positive challenged control, but the vaccination could not prevent the effect of the disease on body weights gain which could be detected after challenge when compared with non vaccinated non challenged group (5).Also, the body weights gain affected in groups (1&2) more than group (3), and this results agree with Sasipreeyajan *et al*. (2012).

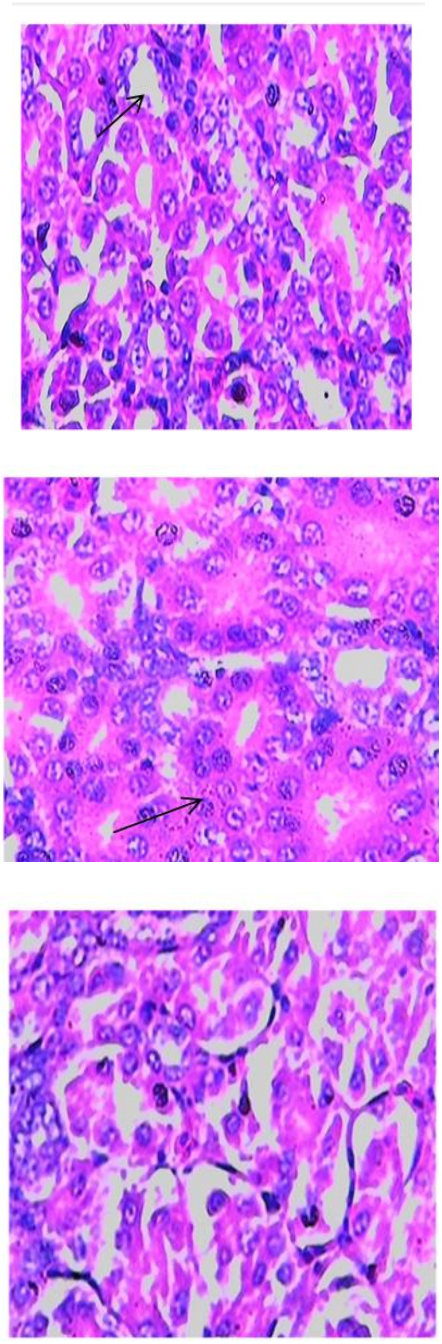


Figure 11.

- Group"1" Left, Kidney of 36-day-old SPF chicks the (IB Primer+ Ma5) vaccinated challenged group (H&EX40), moderate focal interstitial lymphocytic infiltrations (nephritis) and mild hydrobic degeneration.
- Group"2"Middle, Kidney of 36-day-old SPF chicks the (IB Primer+ 4/91) vaccinated challenged group, mild sub acute interstitial lymphocytic infiltrations. (H&EX40).
- Group"3"Right, Kidney of 36-day-old SPF chicks the (IB Ma5+ H120) vaccinated challenged group, extensive acute hydropic degeneration and hemorrhages. (H&EX40).

Live vaccines especially combined variant and classic program are commonly used to control infection with IBV and have been found to be very effective. And worked well in preventing

infection in the challenge of immunity study, although considerable variation exists in the level of protection between different IBV serotypes, this results agree with (Gelb *et al.,* 1981 and 1991). Therefore it is necessary to develop a new IB vaccines, either locally prepared or imported to overcome any new IB serotype that were emerged, through modifying vaccination strategies to make them appropriate to the field situation.

The concept of protectotypes has been suggested to be a valuable one to consider in terms of developing strategies to control IBV infections (Lohr, 1988). The results presented here confirm its value and indicate it to be more relevant in this context than knowing the serotype of a new IB isolate. Rather than spending time determining its serotype, it is probably of more practical relevance in term of control strategies to perform protection studies with the isolate and determine the optimum vaccination programme to protect against it (Cook *et al.,* 1999).

REFERENCES

Abdel-moneim, A.S., Madbouly, H.M., Gelb, JR., Ladman, B.S. 2002. Isolation and identification of Egypt/Beni-Suef/01 a novel genotype of infectious bronchitis virus. *Vet. Med. J. Giza,* 50: 1065-1078.

Ahmed, H.N. 1954. Incidence and treatment of some infectious viral respiratory diseases of poultry in Egypt. Ph.D.Thesis, Fac. Vet. Med. Cairo Univ, Giza, Egypt.

Ali MMZ. 2014. Epidemiological and molecular studies on the current status of infectious bronchitis disease in Egypt. Ph.D. Thesis of poultry Dis. Fac. Vet. Med. Beni-Suef Univ. Egypt.

Avellaneda, G.E., Villegas, P., Jackwood, M.W. and King DJ. 1994. In vivo evaluation of the pathogenicity of field isolates of infectious bronchitis virus. *Avian Dis.,* 38: 589-597.

Baba, T., Masumoto, K., Nishida, S., Kajikawa, T. and Mitsui M. 1998. Harderian gland dependency of immunoglobulin A production in the lachrymal fluid of chickens. *Immunology*, 65: 67-71.

Cavanagh, D. 2003. Severe acute respiratory syndrome vaccine development: Experiences of vaccination against avian infectious bronchitis coronavirus. *Avian Pathol.,* 32:567–582.

Cook, J.K., Chesher, J., Baxendale, W., Greenwood, N., Huggins, M.B. and Orbell, S.J. 2001. Protection of chickens against renal damage caused by nephropathogenic infectious bronchitis virus. *Avian Pathol.,* 30: 423-426.

Cook, J.K., Otsuki, K., Dasilva Martins, N.R., Ellis, MM. and Huggins, M.B. 1992. The secretary antibody response of inbred lines of chickens to avian infectious bronchitis virus infection. *Avian pathol.,* 21: 681-692.

Cook, JKA., Orbell, SJ., Woods, MA. and Huggins, MB. 1999. Breadth of protection of the respiratory tract provided by different live-attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. *Avian Pathol.,* 28: 477_485.

Culling, CF. 1983. Hand book of histological and histochemical techniques. 3rd edition. Butterworths, London, Boston.

Davelaar, F. G., Noorelzij, A. and Vander Donk, J.A. 1982. A study on synthesis and secreation of immunoglobulins by the Harderian gland of fowl after eye-drop vaccination against infectious bronchitis at 1 day-old. *Avian Pathol.,* 11: 63-79.

Davelaar, F.G., Kouwenhoven, B. and Burger, A.G. 1984. Occurrence and significance of infectious bronchitis virus variant strains in egg and broiler production in the Netherlands. *Vet Quarterly,* 6, 114-120.

Gelb, J., Lunt, R.L., Metz, A.L. and Fries, P.A. 1991. Attenuation of avian infectious bronchitis virus by cold adaptation. *Avian Dis.,* 35:847-853.

Gelb, J., Perkins, B.E., Rosenberger, J.K. and Allen, P.H. 1981. Serologic and crossprotection studies with several infectious bronchitis virus isolates from Delmarva-reared broiler chickens. *Avian Dis.,* 25: 655-666.

Hassanein, H.H.A. 2013. Screening on some viral etiologies of kidney affections in broiler chickens. M.V. Sc. Thesis of poultry Dis., *Fac. Vet.Med.,* Cairo Univ., Egypt.

Ignjatovic J. and Galli L. 1994. Structural proteins of avian infectious bronchitis: role in immunity and protectious. In: Coronaviruses. Plenum Press, New York. Pp: 449-453.

Ignjatovic, J. and Sapats, S. 2000. Avian infectious bronchitis virus. Rev. Sci. Off. Int. Epiz.19: 493-508.

Lebdah, M. A., Eid, Amal, A. M., El-Shafey, A. M. 2004. Infectious bronchitis virus infection among meat-type chickens in sharkia province (Egypt). Proc. IV. Int. Symp. On avian Corona-and pneumovirus infections. Rauischholzhausen, Germany, pp. 75-86.

Linda, L.S. 2006. "The Merck Veterinary Manual Online (8th edition). Infectious Bronchitis introduction.

Lohr, J.E. 1988. Differentiation of infectious bronchitis virus strains: In: Proc. lnternl, Symposium on Infectious Bronchitis. E.F. Kaleta and U.Heffels-Redmann (Eds). Rauischholzhausen, Germany. PP.52-53.

Mahgoub, K.M., Bassiouni, A.A., Manalm A.A. and Rabie, S.N. 2010. The Prevalence of Infectious Bronchitis (IB) Outbreaks in Some Chicken Farms. III. Cross Protection of Vaccinated Chickens ersus Field IB Virus.JAS.

Marquardt, W.W., Kadavil, S.K. and Snyder, D.B. 1982. Comparison of ciliary activity and virus recovery from tracheas of chickens and humoral immunity after inoculation with serotype of avian infectious bronchitis. *Avian Dis.,* 26:828-834.

Meir, R., Rosenblut, E., Perl, S., Kass, N., Ayali, G., Perk, S. and Hemsani, E. 2004. Identification of a novel nephropathogenic infectious bronchitis virus in Israel. *Avian Dis.,* 48(3):635-641.

Nix, W.A., Troeber, D.S., Kingham, B.F., Keeler, CL. Jr. and Gelb, J Jr. 2000. Emergence of subtype strains of the Arkansas serotype of infectious bronchitis virus in Delmarva broiler chickens. *Avian Dis.,* 44:568–581.

Reed, L.J. and Muench, H. 1938. A simple method for estimating fifty percent endpoint. *Am. J. Hyg.,* 27: 493-496.

Sasipreeyajan, J., Tawatchai, Pohuang and Nida S. 2012. Efficacy of different vaccination bronchitis virus. *Thai J. Vet. Med.*, 42 (1): 73- 97.

Schalk, A.F. and Hawn, M.C. 1931. An apparently new respiratory disease of baby chicks. *J. Am. Vet. Med. Assoc.,* 78: 413- 422.

Sedeik, E.M. 2005. Studies on infectious bronchitis in chickens. M.V.Sc. Thesis of poultry Dis. Alexandria Univ., Egypt.

Sedeik, E.M. 2010. Further Studies on infectious bronchitis in chickens. Ph.D. Thesis of poultry Dis. Alexandria Univ. Egypt.

Smith, H.W., Cook, J. and Parcell, Z.E. 1985. The experimental infection of chickens with mixtures of infectious bronchitis virus and Escherichia coli. *J. Gen. Virol.,* 66: 777-786. 1985.

Sultan, H.A., Tantawi, L., Youseif, A.I. and Ahmed, A.A.S. 2004. Urolithiathis in white commercial egg laying chickens associated with an infectious bronchitis virus. 6th *Sci. Conf. Egypt Vet.Poult. Assoc.*, 155-169.

Torom, H., Hidalgom, H. and Collingweodselby, A.N. 1994. Lachrymal antibody response of specific-pathogen-free chickens and chickens with maternal immunity after infectious bronchitis virus vaccination. *Preventive Veterinary Medicine*, 18:267-274.

Villegas, P. and Purchase, G.H. 1989. Titration of biological suspension. In: laboratory manual for the isolation and identification of avian pathogens. PP. 186-189. Am. Asso. Avian pathologists. Ames, Iowa, USA.

Wang, C.H. and Huang, Y.C. 2000. Relationship between serotypes and genotypes based on the hypervariable region of the S1 gene of infectious bronchitis Virus. *Arch Virol.,* 145: 291-3.

Winterfield, R.W., Fadly, A.M. and Hoerr, F.J. 1976. Immunity to infectious bronchitis virus from spray vaccination with derivatives of a Holland strain. *Avian Dis.,* 20:42–48.

Worthington, K. J., Savage, C., Naylor, C. J. Wijmenga, W. and Jones, R. C. 2004. An RT-PCR survey of infectious bronchitis virus genotypes in the UK and selected European countries between 2002 and 2004 and the results from a vaccine trial. In: Proceedings of the IV symposium on avian corona and pneumovirus infections. Rauischholzhausen, Germany, pp: 125_133

Yoder, H.W., Drury Jr L.N. and Hopkins, S.R. 1977. Influence of environment on airsacculitis: effects of relative humidity and air temperature on broilers infected with Mycoplasma synoviae and infectious bronchitis. *Avian Dis.,* 21: 195-208.

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