

## Preparation and evaluation of live bivalent infectious bronchitis vaccine in chicken

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**Abstract** : Infectious bronchitis is a highly contagious disease and considered as one of the most important and common poultry diseases. Only few amino acids differences in the S1 protein of infectious bronchitis virus (IBV) may result in poor protection, that make the control of IBV more difficult especially in endemic country such as Egypt. Protection provided by prepared live attenuated bivalent IBV vaccine containing H120 and CR88 IBV strains were compared with those provided by monovalent live attenuated H120 and CR88(D88) vaccines. Assessment of protection following challenge against a local nephropathogenic IBV strain, based on virus reisolation from trachea and kidneys at 3<sup>rd</sup> day post challenge (DPC). Serum IBV enzyme linked immunosorbent assay (ELISA) antibodies geometric mean titres (GMTs) were 5921, 3422 and 8838 for IBV-H120 live attenuated, IBV-CR88 live attenuated and bivalent live attenuated IBV-H120 CR88 vaccines, respectively, where the control group was 349 at the 3<sup>rd</sup> week post vaccination (WPV). Also, these results were confirmed by HI test where the antibody mean titre were 8.80, 9.50, and 10.0 at 3<sup>rd</sup> week post vaccination for CR88, H120, and bivalent H120-CR88, respectively. Our results showed that the combined bivalent IBV-H120 CR88 vaccine was the best protectotype where it gave an excellent immune response at the 3<sup>rd</sup> day post challenge. The protection % was 100% in both trachea and kidney virus reisolation, while it was (100%, 80%) in IBV-H120 vaccine, (60%, 80%) in IBV-CR88 vaccine for trachea and kidney respectively

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### 1. Introduction

Avian infectious bronchitis virus (IBV) is a major cause of economic losses in the poultry industry. Since the first description of infectious bronchitis (IB) in the late 1930s (**Schalk and Hawn, 1931**), infectious bronchitis virus (IBV) has been involved in respiratory disease and poor egg laying performance in chickens. Also, IBV was associated with nephritis which can be induced through viraemia following infection of the respiratory tract (**MacDonald and McMartin, 1976**). The prevalence of IBV induced nephritis may be affected with the nephrotropism of IBV strain and the humoral immune response status of the bird (**Chubb, 1973**). IBV replicates primarily in the respiratory tract and also in some epithelial cells of the gut, kidney and oviduct (**Cavanagh, 2003**). IBV is a virus member of genus Coronavirus, family Coronaviridae, order Nidovirales (**Cavanagh and Naqi, 2003**). The virus posses a positive standard RNA genome that encodes phosphorylated nucleocapsid protein (N), membrane glycoprotein (M), spike glycoprotein (S) and small membrane protein (E). Diversity in S1 probably results from mutation and recombination (**Song et al., 1998**).

Antigenically different serotypes and newly emerged variants from field chicken flocks sometimes

cause vaccine breaks (**Gelb et al., 1991, Pohuang et al., 2009**).

In Egypt, isolates related to Massachusetts, D3128, D274, D-08880, 4/91 and the novel genotype were isolated from different poultry farms (**El-Kady, 1989; Sultan et al., 2004 and Susan et al., 2010**). The commonly used IBV attenuated vaccine is H120 while the Mass41 (M41) strain is commonly used in inactivated vaccines, but in many cases the renal damage was observed in IB-vaccinated flocks, suggesting that the currently used IB vaccination procedures may not be providing adequate protection.

**Cook et al. (1999)** showed that broad protection of the respiratory tract against heterologous IB serotypes could be achieved using a combined vaccination incorporating the IB Ma5 and 4/91.

The objective of the present work was to prepare a bivalent infectious bronchitis vaccine containing H120 and D88 strains of IBV and to determine the protection percent of vaccinated birds against challenge with an IBV capable of inducing infection.

### 2. Material and Methods

#### 1. Chicken embryos and chicks:

Specific pathogen free (SPF) chicks, and emryonated chicken egg (ECEs) were purchased from Ministry of Agriculture, Specific Pathogen Free Farm,

Koum Osheim SPF (specific pathogen free). embryonated chicken eggs (9-11) day old were used for propagation and titration of live IBV strains and vaccines. One day old SPF chicks were housed in positive pressure stainless steel isolation cabinets, and used for vaccines evaluation.

## 2. Virus and vaccines:

### \* local isolated IBV strain:

Local nephropathogenic strain IBV was isolated from broiler chickens 24 day old at Dakhalia, with a history of respiratory and renal signs, and vaccination against IBV. The local isolate was matched for 96% with isolated strain Egypt/F/03 strain, with accession No.DQ487085 (NCBI) the isolated strain titre was  $10^6$  EID<sub>50</sub>/ml. It was kindly obtained from Dr.Khaled Mahgoub, National Research Center (N.R.C) Dokki.

### \* H120 IBV-vaccine:

Izovac H120 live attenuated virus vaccine of IBV, strain Massachusetts H120 with a titre of 8.5 log<sub>10</sub> EID<sub>50</sub>/ml was kindly obtained from IZO, s.p.a., Italy.

### \* D88 IBV vaccine:

Gallivac IB88 live attenuated virus vaccine of IBV Coronavirus, strain CR88121 with a titre of 8.3 log<sub>10</sub> EID<sub>50</sub>/ml was kindly obtained from Merial SAS France.

## 3. Tryptose phosphate broth:

It was used in cultivation of tracheal swabs for reisolation of viral shedding.

## 4. Titration of live IBV vaccines:

The titre of live IBV vaccines was carried out for H120 and D88 vaccines according to OIE (2008) using SPF (ECE) 9-11 day old via the allantoic cavity. The surviving (ECE) were examined for lesions of IBV (curling and dwarfing) of the embryos, 5-7 days post inoculation.

## 5. Identity test:

It was carried out for both H120 and D88 strains for detection of S1 gene in the vaccinal strains using specific primers (OIE, 2008)

### \* Genomic RNA extraction:

The genomic RNA was extracted from the reconstituted vaccine using EZ.10 Spin column viral RNA extraction kit cat # (VT82112) according to the manufacturer instruction.

### \* RT-PCR:

cDNAs were produced from the extracted RNAs using RT Kit (Sib Enzyme Ltd. # E317), then amplification of the produced cDNAs were done according to PCR kit manufacturers (AmlI Taq Gold DNA Polymerase kit # N808-0240, Roche). The PCR products were observed on 1% agarose gel in electrophoresis test.

## 6. Preparation of the bivalent IBV live vaccine:

It was carried out according to OIE (2008).

- IB virus propagation: Preparation of the live monovalent liquid vaccine separately for each of the two strains H120 and CR88 (Cunningham, 1973).
- Titration of IB strains: The titre of the virus used was 8.5 and 8.3 log<sub>10</sub> EID<sub>50</sub>/ml for the H120 strain and CR88 strain respectively.
- Mixing equal volume of the harvested allantoic fluid 40 hours post ECE inoculation of the two IBV strains H120 and CR88 and add 20% skimmed milk as stabilizer to the mixture.
- Lyophilization of bivalent live vaccine containing H120 and CR88 strains.
- Quality control as sterility and safety tests was applied on the prepared vaccines according to OIE (2008).

## 7. Potency and efficacy test: (OIE, 2008)

SPF chickens, seven days old, were vaccinated intra ocular (I/O) with one field dose of the commercial monovalent and prepared bivalent IB vaccines. The blood serum samples were weekly collected 3 weeks post vaccination. The vaccinated and non-vaccinated control groups were challenged at 3<sup>rd</sup> week post vaccination via oculonasal route with IBV challenge strain (local nephropathogenic IBV strain). The challenge virus dose was adjusted to 0.1 ml containing  $10^4$  EID<sub>50</sub>/bird. All the birds were observed daily for 10 days post challenge (PC). Three days PC, morbidity and mortality rates were recorded for vaccinated and non-vaccinated groups till the end of the observation period, to measure the protection %.

The assessment of viral shedding due to replication of IB challenge virus was performed (OIE, 2008) through collection of tracheal swabs in culture media containing antibiotic mixture on 3<sup>rd</sup> day post challenge from live infected control group as well as the vaccinated chickens using 9-11 days old SPF (ECE).

## 8. Experimental Design:

In this study, one hundred, 7 days old SPF chickens were used to evaluate the efficacy of prepared live monovalent and bivalent IBV vaccines. The birds were divided into 4 experimental groups (25 birds/each). All groups were vaccinated with the prepared vaccines as shown in Table (1).

**Table (1)** Groups vaccinated with prepared vaccines

Groups	No. of birds	Type of used vaccine
1	25	Vaccinated with live monovalent H120 IB vaccine
2	25	Vaccinated with live monovalent CR88 IB vaccine
3	25	Vaccinated with live bivalent H120-CR88 IB vaccine
4	25	Non-vaccinated control group

\* Blood serum samples were collected weekly.

- \* 15 birds of each group were challenged 3 weeks post vaccination against the local nephropathogenic IBV strain.

#### 9. Enzyme linked immunosorbent assay (ELISA):

It was carried out for estimation of antibodies against IBV vaccines according to kit manufacture (Biochek Co.).

#### 10. Haemagglutination inhibition (HI):

HI test was carried out according to **Munir et al. (2012)**. Haemagglutinating antigens were prepared from allantoic fluid harvested from IBV-inoculated SPF eggs. The concentrated antigens were treated with trypsin and used as HA antigens according to **Mahmood et al. (2004)**.

#### 11. Virus reisolation from tracheal swabbing and kidneys:

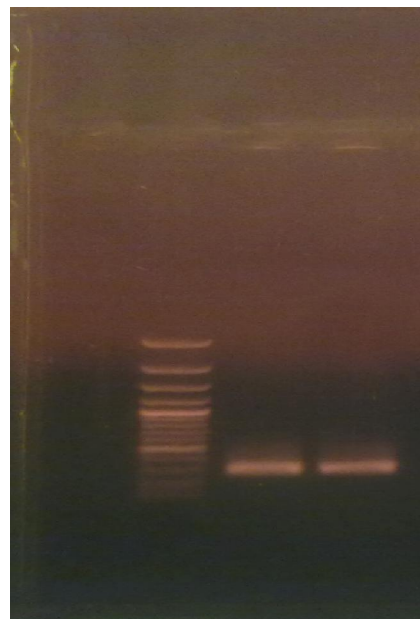
The test was carried out according to **(Gelb and Jackwood, 1998)**.

- \* Tracheal swabbings were placed in 3 ml tryptose phosphate broth (TPB) with antibiotics (10,000 IU penicillin and 10,000 mg streptomycin/ml) and stored at -70°C until used for virus isolation.
- \* Kidney homogenates were made with tissue collected approximately 1gm of tissue was placed in 3ml TPB with antibiotics. Homogenates were frozen and thawed three times and clarified by centrifugation at 2000 xg for 5 minutes. The supernatant was collected and stored at -70°C until used.
- \* Tracheal swabbings and kidney homogenates were inoculated into 10-11 days old SPF embryonated chicken eggs via allantoic sac.
- \* Seven days post inoculation, the embryos were evaluated for IBV lesions such as stunting, curling and kidney urates.

### 3. Results and Discussion

Infectious bronchitis virus (IBV) is a common, highly contagious disease of respiratory and urogenital tract of chickens. Young chickens typically develop respiratory disease whereas adult hens experience reduced egg production and shell quality with or without coughing, sneezing and rales (**MacDonald and McMartin, 1976**). Some strains of the virus have an affinity for kidney and produce nephrosis-nephritis in young birds or urolithiasis in layers. Control of the disease is difficult because many field strains differ antigenically (**Cavanagh, 2003**). Our research describes some of our recent findings and discusses possible approaches to control.

The IB strains were identified using RT-PCR as shown in photo (1). Amplification of 300bp fragments of S1 gene were produced from all IB vaccines (**Gelb and Jackwood, 1998**).



**Photo (1): The amplification of the 300bp fragment of the S1 gene of IB virus of both vaccine batches**

**Table (2): The antibody response to different IB vaccines using ELISA test**

Test group	Type of used vaccine	Geometric mean antibody titre		
		Weeks Post Vaccination		
		1	2	3
1	IB CR88 live vaccine	889	3270	3422
2	IB H120 live vaccine	833	2254	5921
3	Combined IB CR88-H120 live vaccine	629	2811	8838
4	Non-vaccinated control	364	354	349

**The minimum positive level serum is equal or more than 3000 in ELISA antibody titre for IB living vaccine according to Kit manufacture.**

The antibody response of bivalent and monovalent vaccines was determined by enzyme linked immunosorbent assay. Table (2) revealed that group received CR88 vaccine gave antibody value alternates between 889, 3270 and 3422 in weeks 1, 2, 3 respectively. Regarding to group received H120 vaccine, the ELISA antibody titers increased gradually from 1<sup>st</sup> week (833) till record the highest level in 3<sup>rd</sup> week (5921).

For group which received bivalent CR88 and H120 vaccine, the antibody titres were 629, 2811 and 8838 increased 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> weeks post vaccination respectively. These results were in agreement with **Sasipreeyajan et al., (2012)** who said that the immune response of chickens vaccinated with the attenuated IB

vaccines were increased from the 1<sup>st</sup> wpv and reached the maximum level at 3<sup>rd</sup> wpv.

Also, the mean HI titres of different IB vaccines used in the test at (1-3) weeks post vaccination were (5.2-8.0-8.8) for IB CR88 and (6.4 – 9.12 – 9.5) for IB H120 while it was (6.6 – 9.5 – 10.0) for bivalent IB

CR88-H120, as shown in table (3) that agree with **Macpherson and Feest (1978)**, **MacDonald et al. (1981)**, **Mahmood et al. (2004)** and **Terregino et al. (2008)** who said that the bivalent IB vaccine was considered the best one on giving the highest antibody titres.

**Table (3): The antibody response to different IB vaccines using HI test**

Test group	Type of used vaccine	Mean antibody Titre		
		Weeks Post Vaccination		
		1	2	3
1	IB CR88 live vaccine	5.20	8.00	8.80
2	IB H120 live vaccine	6.40	9.12	9.50
3	Combined IB CR88-H120 live vaccine	6.60	9.50	10.0
4	Non-vaccinated control	0	0	0

**Table (4): Re-isolation results at three days post challenge against local field isolate of nephropathogenic IBV strain**

Test group	Type of used vaccine	Clinical signs	Kidney		Trachea	
			Virus isolation		Virus isolation	
			No. of +ve samples*	Protection %**	No. of +ve samples	Protection %
1	IB CR88 live vaccine	2/5	1/5	80 %	2/5	60 %
2	IB H120 live vaccine	2/5	1/5	80 %	0/5	100 %
3	Bivalent live vaccine IB CR88-H120	0/5	0/5	100 %	0/5	100 %
4	Non-vaccinated control	5/5	5/5	0 %	5/5	0 %

\* Number of positive samples / Total number of samples

\*\* Protection after the reisolation of the virus.

#### **NB:**

1. Clinical signs started to appear at 3<sup>rd</sup> day post challenge in the form of mild to moderate rales in non-vaccinated challenged group revealed 0%.
2. The protection% is considered valid if the challenge virus is isolated from not less than 80% of control group and less than 20% of vaccinated birds according to **European Pharmacopeia Reference Standards (Council of Europe, 2007)**.

Table (4) showed the results of protection% of chickens after proceeding the challenge test using viral reisolation from both kidney and trachea. The table implies that CR88 vaccine protected the kidney with 80% but it protected trachea with 60%. In case of H120 vaccine, the protection was 80% for the kidney and 100% for trachea, while in bivalent vaccine it was 100% protection for both kidney and trachea. The non vaccinated control group was 0%. The above data indicates that bivalent vaccine is highly effective and could protect the chickens from being infected upon the exposure to different strains of IBV. These results agree with **Gelb et al. (1989)** who suggest that cross protection produced by some IBV serotypes against antigenically unrelated strains is well known and the combination of strains may produces complete cross-protection against variant serotypes. Also agree with **MacDonald et al. (1981)** who reported complete protection against challenge with nephrogenic H52 strain of IBV and prevented the multiplication of virus

in the kidney after vaccination with the H120 strain of infectious bronchitis virus (IBV) and agree with **Cook et al. (2001)** who reported that Ma5 vaccine alone provided poor protection but in combination with 4/91 strain both protected well. The results are also in agreement with **Thompson et al (1997)** and **Sasipreeyajan et al. (2012)**.

So, it could be concluded that the bivalent vaccine was safe, potent and induce high levels of antibody titres, in addition to giving a high protection in vaccinated chickens.

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