Comparative study between prepared bivalent IB inactivated vaccine and commercial one

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A B S T R A C T

Infectious bronchitis is a highly contagious disease, considered as one of the most important poultry diseases and it causes huge economic losses in poultry industry due to its effect on broiler growth and egg production quantity and quality in laying hens, Egyptian poultry farms suffer from frequent respiratory disease outbreaks associated with IBV variant 2 strains. Inactivated vaccines are important component in vaccination program of layers and breeders for egg production and also provide protection against early infection through maternal immunity in progeny. In this study, a newly prepared inactivated classical and variant 2 vaccine was compared for its field efficacy with commercial (ND&IB) in layer chickens. Vaccinated chickens from both groups were evaluated against both homologous and heterologous challenge in comparison to control unvaccinated groups. Four estimated parameters were applied in this experiment, humoral immunity after vaccination and challenge by Enzyme linked immunosorbent assay (ELISA), egg production percentage, detection of challenge strains by real-time RT-PCR, and histopathological findings after challenge from tracheal and oviduct samples. The results revealed that the prepared vaccine give higher protective antibody titers in serum and better egg production after challenge than commercial inactivated (ND&IB) one . The detection of IB viruses shedding was limited to trachea regarded its effect on oviduct except only in control groups. The current study demonstrates the effectiveness of locally prepared bivalent IBV vaccine in commercial layers; also combination of inactivated variant and classical IBV strains confers broad protection against both homologous and heterologous challenge in IBV endemic areas.

Key words: *IBV, layer chickens, inactivated vaccine, variant 2*
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1. INTRODUCTION

Avian infectious bronchitis virus (IBV) is a highly contagious pathogen of commercial poultry with a predilection for the upper respiratory tract and it cause reduction in production, in addition to causing disease in kidneys resulted in nephritis in young chicken .In adult, IBV effects on reproductive tract and produces irreparable damage to oviduct and production of abnormal eggs. Lesions in infected birds include the degeneration of renal and ciliated respiratory epithelia (Cavanagh, 2007; Cook *et al*., 2012; Jackwood, 2012; Jackwood and de Wit, 2013).IBV is a single-stranded enveloped RNA virus that belongs to the genus Gammacoronavirus, family Coronaviridae (Cavanagh, 2005). In Egypt, many strains were isolated from different poultry farms,(e.g.) Massachusetts D3128, D274, D08880, 793B, variant 1 and 2 strains (Abdel-Moneim *et al*., 2006 ; Zanaty *et al*., 2016a) and IS/1494/06 nephropathic IBV strains were isolated from poultry farms (Susan *et al.*, 2010). Recently studies classified the isolated IBV strains into Egyptian variant 1 and Egyptian variant 2 based on the S1 hypervariable region 3 sequence analysis (Zanaty *et al.*,2016a and b), the IBV variant 2 is the most predominant serotype in Egypt which currently causing massive losses in broiler, layer, and breeder sectors (Susan *et al*., 2011; Abdel-Moneim *et al*., 2012). Many IBV serotypes and genotypes are emerging worldwide that made the

disease difficult to be controlled, different vaccination programs using imported vaccines which failed to protect our flocks from field challenge. Recently protection studies of live attenuated variant strains indicated that using of local strain vaccine can provide better protection against IBV challenge (Lee *et al*., 2010; Zhao *et al*., 2015; Ahmed *et al*., 2018).

Both live attenuated and inactivated vaccines are used in IBV immunization programs, however inactivated vaccines provide protection against drop in egg production which not always be given by live vaccination (Box *et al*, 1980). Emergence of new "variant" strains require rapidly preparation of inactivated autogenous vaccines for controlling IB in laying birds without the risks of using a live variant vaccine that could spread and potentially produce the disease. Inactivated variant vaccines may offer better protection against challenge with the virulent variant IBV than inactivated vaccines containing standard serotypes such as Massachusett and Connecticut. It is recommended to continue studying of different IB vaccination protocols and to prepare an autogenous vaccine from this variant to help in controlling the disease and to reduce the economic loss in the Egyptian chicken industry. (Ladman *et al* ., 2002; Jackwood, 2012 ; Zanaty *et al* .,2013).The present study was directed to assess the field effectiveness of locally prepared bivalent IBV inactivated vaccine in comparison to commercial (Newcastle disease & Infectious bronchitis(ND & IB) inactivated vaccine and its protective effect against both homologous and heterologous challenge.

2. MATERIALS AND METHODS:

2.1. Experimental host:

Two hundred and thirty chickens' aged, 17 week old red lohman chickens were purchased from field layer chicken farm, Meet Kenana, EL-Qalubia Governorate. They were floor reared, fed on prepared poultry ration and kept under strict hygienic measures throughout the experiment. With vaccination history of the flock was established from the owner, for IB disease, the vaccine commonly used was the live vaccine based on eye drop (hitchner IB H120) at 7 days of age and inactivated (ND & IB) vaccine at 30 day of age.

2.2. IB vaccines:

2.2.1. Bivalent inactivated IBV vaccine

Containing the variant 2 IBV strain VACSERA/2014, Genbank accession no; KP279998 and classical strain H120 IBV VACSERA/2014, Genbank accession no; KP279995 (Reda *et al.,* 2019). Infective dose contain $10^{8.5}$ 0.5 cm /bird with S/C route

2.2.2. Commercial combined inactivated vaccine

Containing the following viral strains (ND Clone 30& IB M41) with batch number (59024), Intervet International, Holland containing 500 ml (1000 doses).

2.3. IB challenge strains:

2.3.1. Homologous challenge strains:

Strains of classical IBV (Chicken / Egypt/ KP279995 / vacsera / 2014) and variant 2 IBV (Chicken / Egypt KP279998 / vacsera / 2014) (Reda *et al.,* 2015).

2.3.2. Heterologous challenge strain:

Virulent strain of IBV (Chicken/Egypt /KP729422/VSVRI/2013) was kindly obtained from Veterinary Serum and Vaccine Research Institute.

2.4. Sampling:

Collected blood samples were taken with weekly interval after vaccination for 7 weeks and separated by centrifugation to obtain serum from each collected samples at 5000 rpm/15min.

2.5. Enzyme–linked Immunosorbent Assay (ELISA) kit:

ELISA Kit was obtained from Biocheck poultry immune assays for estimation of humoral immunity by IBV antibody test kit (CK119) with

serial No. F69371 and product code: 6020. All procedures were conducted according to manufacturer's instructions.

2.6. Real-time Reverse transcriptase chain reaction (RRT-PCR) according to (Meir *et al.*, 2010):
Extraction of viral RNA carried out by QIA amp Viral RNA Mini Kit (QIAGEN) with catalogue No. (52904). All procedures were conducted according to manufacturer's instructions. Preparation of master mix was conducted using Quanti Tect probe RT-PCR with catalogue no. (204443). Primers and probes used were supplied from Metabion (Germany) as shown in table (1).

2.7. Reagents for histopathology

According to (Bancroft *et al*., 1977). It was carried for recording changes in trachea and oviduct.

2.7. Statistical analysis:

For presentation of results, the means and standard errors of the mean (SEM) were calculated. Analysis of variance (ANOVA) was performed using the Statistical Analysis System software (SPSS, 2004), results were considered statistically significant when ($P<0.05$).

2.8. Experimental design:

Two hundred and thirty Red Lohman layer chickens aged (17week old) were divided into 3 groups (group A and group B contain 100 chickens for each, while group C contain 30 chickens. At 19 week old , the groups were treated with different manner as first group (A), birds were vaccinated with 0.5 ml S/C per bird of locally prepared bivalent IB inactivated vaccine. The second group (B), birds were vaccinated with commercial combined (ND &IB) inactivated vaccine and third group (C) were kept as unvaccinated control group. Blood samples were taken at 2, 3, 4, 5 ,6 and 7 weeks post vaccination and egg performance parameter was evaluated for 7 week from all groups

2.8.1. Challenge test:
For evaluation of protection against homologous and heterologous challenge after one month from vaccination, groups A and B were divided into 2 subgroups (G1,G2 and G3,G4) respectively, while group C were divided into 3 subgroups (G5,G6,G7). Subgroups G1, G3 and G5 challenged with homologous strain as the same of prepared vaccine and subgroups G2, G4 and G6 challenged with heterologous strain, while birds of subgroup 7 kept as control negative without any treatment as shown in table (2). Egg performance and mean antibody titers were monitored after challenge for two weeks also the detection of viral shedding from both vaccinated and unvaccinated control groups was done by collection of the tracheal swabs at interval 3rd, 5th and 7th days post challenge while oviduct samples were collected at interval 7th, 10th and 14th days post challenge. The histo-pathological changes were recorded for 2 weeks post challenge in different groups.

3. RESULTS

3.1. Assessment of humoral antibody levels:
3.1.1.Mean antibody titers after vaccination :

At first 3 weeks after vaccination, there was mild numerical difference in titers of the first group in comparison to group B and group C . At the 4th week, the vaccinated groups (GA and GB) showed significant increase in the mean antibody titers ($P<0.05$) (1202.8±7.414 and 1030.6±8.307) respectively in comparison to control (–ve) group (475.80±15.99$^{b)}$. Group A showed higher antibody titers at 5th week from vaccination (1260.0±3.67^{a}) followed by group B (1002.6±1.22^{b}) in comparison to control (–ve) group (244.3±35.4 ^{c}) as shown in table (3) and figure (1).

3.1.2.Mean antibody titers after challenge :

Heterologous challenge sub groups (G2 and G4) showed numerical drop in the mean antibody titers (921.3±13.51^{a} and 787.7±26.20^{a}) than homologous challenge one (G1and G3) (980.0±10.56^{a} and 951.6±11.74^{a}) respectively. After 2 weeks from challenge, as a same homologous subgroup (G1 and G3) recorded higher mean of antibody titers (884.23±13.01^{b} and 830.6±71.41^{b}) than heterologous challenge sub groups (G2 and G4) (792.2±14.75^{b} and 581.8±97.47^{b}) respectively , it was obvious that

heterologous challenge strain had marked effect on the mean antibody titer that was prominent in G4 than G2 .After two weeks of challenge ,homologous and heterologous challenge sub groups (G5 and G6) recorded the highest level of antibody titer (1006.9±71.12[a] and 1080.6±64.01[a]) respectively as a result of infection with IBV rather than vaccination in comparison to control (-ve) sub group (7) as shown in table (4) and figure(2). From analyzed data, vaccination with prepared bivalent IBV confers higher protective antibody titers against homologous and heterologous challenge than imported combined (ND&IB).

3.2.1. Egg performance parameter after vaccination in three groups:

Three groups were evaluated for egg production after vaccination , control group (C) showed significantly ($P < 0.05$) increased in egg production at weekly interval in comparison to vaccinated groups. However , the percent of egg production was numerically higher in group (A) in comparison to group (B) after vaccination as shown in table (5) and figure (3).

3.2.2. Egg performance parameter after challenge in the seven sub groups:

Egg production was evaluated in 7 subgroups during the two weeks of challenge. At 1st week post challenge, there is obvious decrease in heterologous (G2 and G4) as (22% and 21%) respectively than homologous challenge subgroups (G1 and G3) as (33% and 25%) similar finding results in control challenge subgroups (G5 and G6) but egg production not affected in control negative sub group (G7). At the end of 2nd week from challenge, egg production recorded marked difference in vaccinated challenged subgroups than control ones. Heterologous challenge had progressive effect on the oviduct of control sub group G6 (10%) than sub group G5 (20%) , on the other hand vaccinated with homologus challenge subgroups (G1 and G3) recorded gradual recovery in egg production than heterologous challenge sub groups (G2 and G4) as in table (6) and figure (4). From recorded results, locally vaccine showed better effect on egg production at the time of challenge.

3.3.Detection of viral shedding after challenge in vaccinated and control sub groups:

3.3.1.Tracheal samples:

Viral shedding could be detected at 3 days post challenge in the trachea of vaccinated and unvaccinated challenged sub groups, with higher viral titer in G5 and G6 unvaccinated control subgroups. At 5 days post challenge, trachea from homologous challenge subgroups G1 and G3 showed (–ve) reaction, while heterologous subgroups G2 and G4 had lower viral titers than control (+) G5 and G6. Birds in all vaccinated subgroups recorded (-ve) reaction at 7 days post challenge whereas unvaccinated control subgroup G5 and G6 showed (+) reaction as shown in table (7) and figure (5), (6) and (7). Vaccination with prepared inactivated IBV vaccine had good protection against both homologous and heterologous challenge with decreasing viral shedding rate in vaccinated birds in comparison to unvaccinated ones.

3.3.2. Oviduct samples:

Viral shedding could first be detected at 7 days post challenge in the oviduct of unvaccinated challenged subgroups. At 14 days post challenge, oviduct from both sub group (G5 and G6) had higher viral titers than the first week from challenge but birds in all vaccinated subgroups (G1,G2,G3,G4) had (-ve) reaction in two times of viral detection as shown in table (8) and figure (8) and (9).

3.4.Histopathological findings

The effect of viral shedding in both vaccinated and unvaccinated subgroups can be demonstrated by using histopathological technique, heterologous challenge subgroup had marked changes on both trachea and oviduct samples than homologous challenge subgroup. The histopathological findings of trachea revealed presence of hyperplasia of lining epithelium with activation of mucous glands and mononuclear cells infiltration in lamina propria and congested blood vessels in the control group. Collected oviduct samples revealed loss of cilia of luminal epithelium, lymphocytic cellular aggregates in lamina propria,

congested blood vessels and intermuscular edema mixed with few lymphocytes in tunica muscularis. Excessive mucus exudates on the mucosa and hydropic degeneration of glandular epithelial cells characterized by large pale vacuolated cytoplasm as showed in the following figures (10,11,13,14 and 15) .

Table (1): showing oligonucleotide Primers and probes for IBV

Virus	Gene	Primer/ probe sequence 5'-3'	Ref
IB	N	AIBV-(Forward) ATGCTCAACCTTGTCCCTAGCA AIBV-(Reverse) TCAAACTGCGGATCATCACGT AIBV-TM (Probe) [FAM]TTGGAAGTAGAGTGACGCCCAAACTTCA [TAMRA]	Meir *et al*., 2010

Table (2): Experimental design of vaccination and challenge with both homologous and heterologous strains for layer chickens

Groups	No of birds/subgroup		Age at vaccination	Treatment	Age at challenge	Type of challenge
A	G1	50	19 week old	prepared	24 week old	G1 → Homologous
	G2	50	19 week old	vaccine	24 week old	G 2 → Heterologous
	G3	50	19 week old	commercial	24 week old	G 3 → Homologous
	G4	50	19 week old	combined (ND&IB) vaccine	24 week old	G 4 → Heterologous
C	G5	10	-	Unvaccinated	24 week old	G 5 → Homologous
	G6	10	-	control	24 week old	G 6 → Heterologous
	G7	10	-		Not challenged	G 7 → G Control (-)

Table (3): The Mean antibody titers of vaccinated groups and control group after vaccination using ELISA test.

Group/ weeks	Group A	Group B	Group C
Before vaccination	855.27±6.37 9[a]	855.27±6.379[a]	855.27±6.379[a]
2 weeks post vaccination	791.20±1.872[a]	730.37±1.609[a]	703.60±10.32[a]
3 weeks post vaccination	843.4±2.171[a]	750.6±1.871[a]	569.8±13.92[a]
4 weeks post vaccination	1202.8±7.414[a]	1030.6±8.307[a]	475.80±15.99[b]
5 weeks post vaccination	1260.0±3.67[a]	1002.6±1.22[b]	244.3±35.4[c]

Table (4): The Mean antibody titers of vaccinated groups and control group after challenge using ELISA test.

	G1	G2	G3	G4	G5	G6	G7
(1st week challenge)	980.0± 10.56[a]	921.3± 13.51[a]	951.6± 11.74[a]	787.7± 26.20[a]	933.4± 91.84[a]	841.6± 11.77[a]	455.33± 16.03[b]
(2nd week challenge)	884.23± 13.01[b]	792.2± 14.75[b]	830.6± 71.41[b]	581.8± 97.47[b]	1006.9± 71.12[a]	1080.6± 64.01[a]	437.0± 26.39[c]

Table (5): Egg production percentage in 3 groups per week after vaccination

week	Group	Group A	Group B	Group C
First week post vaccination		13 %	10 %	16%
Two week post vaccination		23 %	21 %	28 %
Three week post vaccination		52 %	48 %	56 %
Four week post vaccination		74 %	65 %	80 %
Five week post vaccination		60 %	52 %	78 %

Table (6): Egg production percentage in 7 subgroups per week after challenge

	G1	G2	G3	G4	G5	G6	G7
1st week from challenge	33%	22%	25%	21%	25%	15%	40%
2nd week from challenge	34%	25%	27%	22%	20%	10%	40%

Table (7): Detection of IBV nucleic acid using Real-time PCR in tracheal samples of vaccinated and unvaccinated sub groups.

Sample type	Group	Days post infection	Result	CT	Virus titer (EID50/ml)
Trachea	G 1	3 days	Positive	24.18	2.268×10^3
	G 2		Positive	23.67	1.361×10^3
	G 3		Positive	24.71	4.134×10^3
	G 4		Positive	22.86	4.064×10^3
	G 5		Positive	21.08	2.586×10^4
	G 6		Positive	20.39	5.873×10^4
	G 7		Negative	-	-
Trachea	G 1	5 days	Negative	-	-
	G 2		Positive	27.52	3.094×10^2
	G 3		Negative	-	-
	G 4		Positive	28.09	2.855×10^2
	G 5		Positive	24.96	1.797×10^3
	G 6		Positive	22.18	1.702×10^4
	G 7		Negative	-	-
Trachea	G 1	7 days	Negative	-	-
	G 2		Negative	-	-
	G 3		Negative	-	-
	G 4		Negative	-	-
	G 5		Positive	27.09	4.158×10^2
	G 6		Positive	26.57	8.171×10^2
	G 7		Negative	-	-

Table (8): Detection of IBV nucleic acid using Real-time PCR in oviduct samples of vaccinated and unvaccinated subgroups.

Sample type	Group	Days post infection	Result	CT	Virus titer (EID50/ml)
Oviduct	G 1	7 days	Negative	-	-
	G 2		Negative	-	-
	G 3		Negative	-	-
	G 4		Negative	-	-
	G 5		Positive	28.11	2.063×10^2
	G 6		Positive	26.59	8.058×10^2
	G 7		Negative	-	-
Oviduct	G 1	14 days	Negative	-	-
	G 2		Negative	-	-
	G 3		Negative	-	-
	G 4		Negative	-	-
	G 5		Positive	23.73	4.185×10^3
	G 6		Positive	22.28	1.589×10^4
	G 7		Negative	-	-

Fig (1): The mean antibody titers in all groups after vaccination using ELISA test

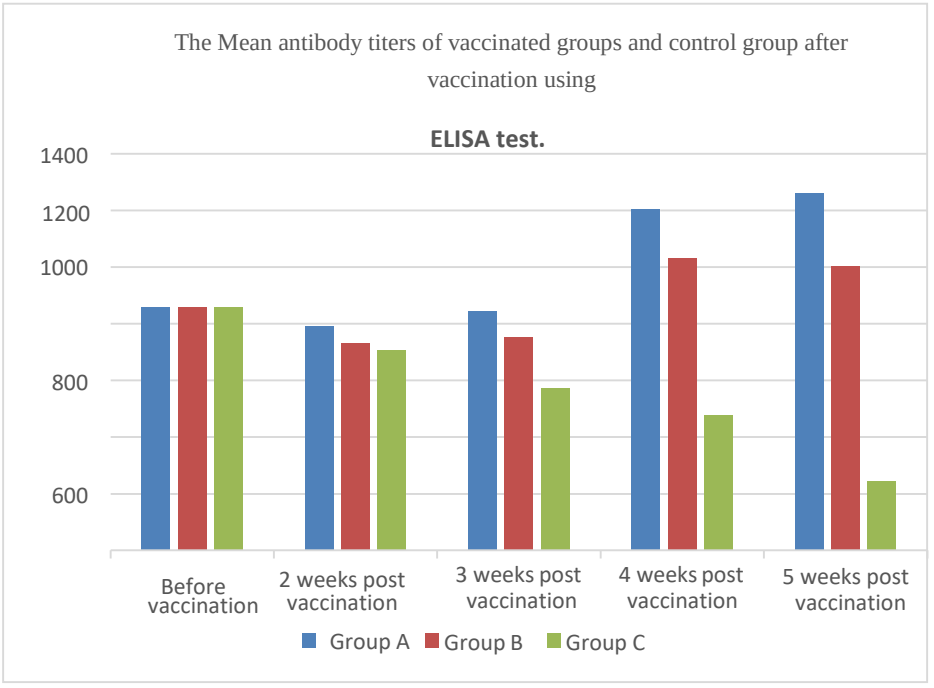


Fig (2): The mean antibody titers in all sub groups after challenge using ELISA test

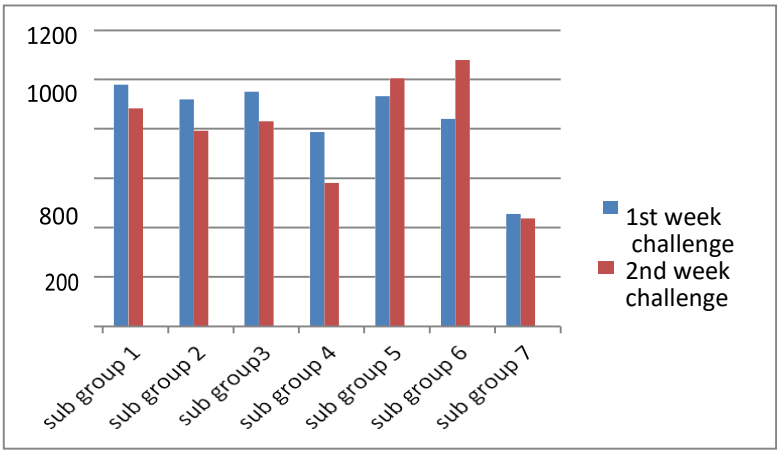


Figure (3): Egg production percentage in 3 groups per week after vaccination

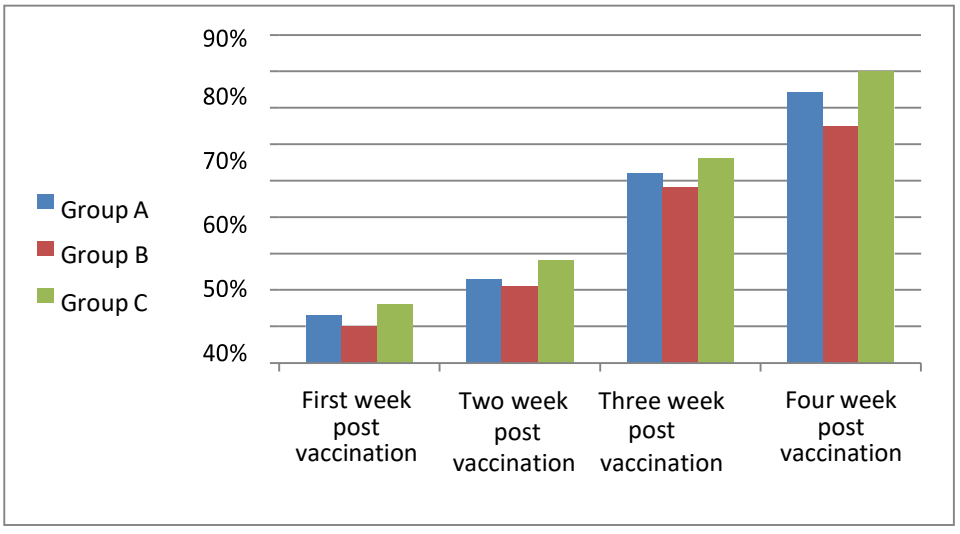
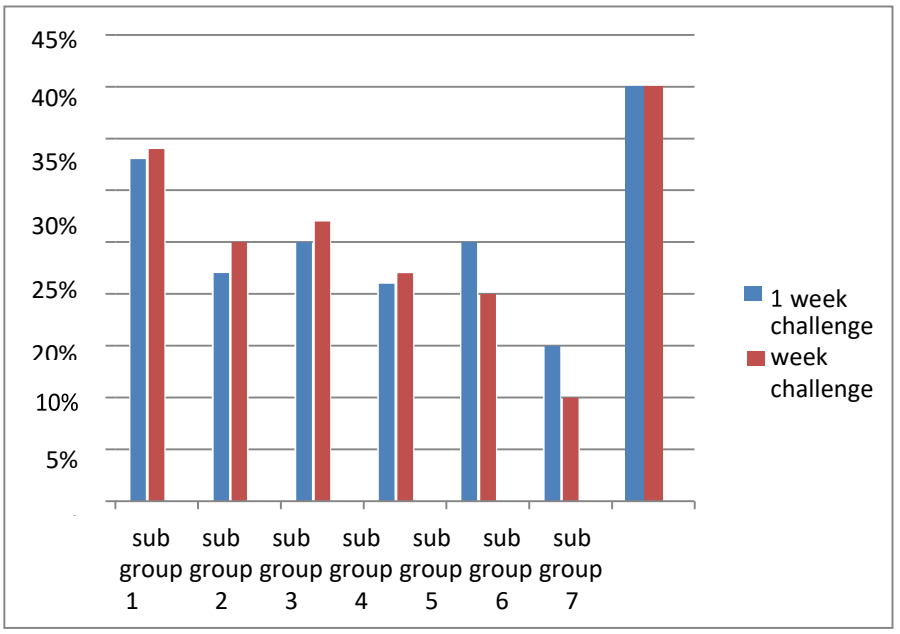


Figure (4): Egg production percentage in seven subgroups per week after challenge



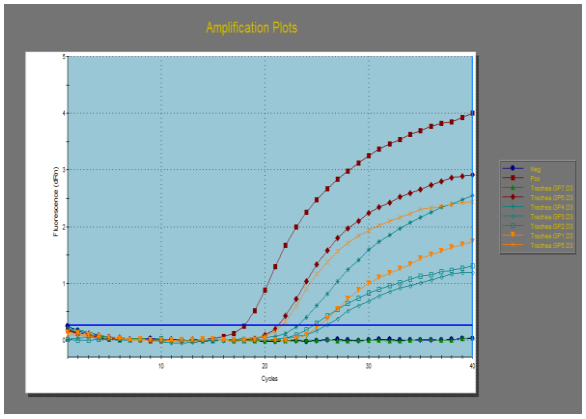


Fig (5):Real time -Polymerase chain reaction curve Showing detection of IBV nucleic acid in trachea at 3 days post vaccination

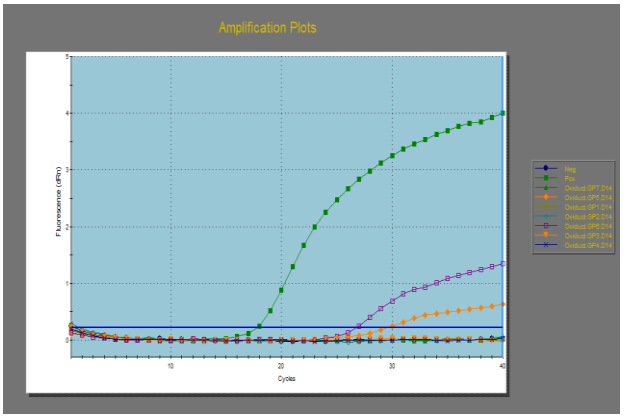


Fig (6): Real time -Polymerase chain reaction curve showing detection of IBV nucleic acid in trachea at 5 days post vaccination

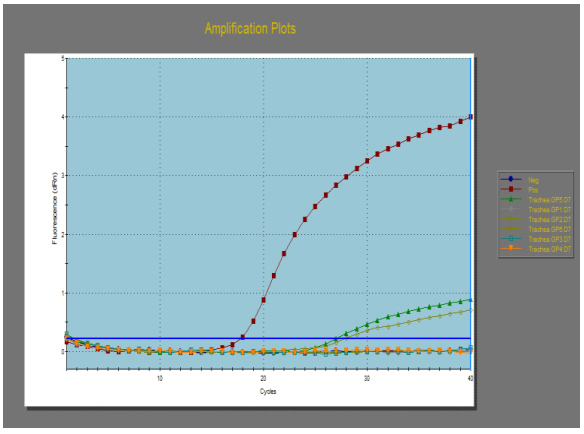


Fig (7): Real time –Polymerase chain reaction curve showing detection of IBV nucleic acid in trachea at 7 days post vaccination

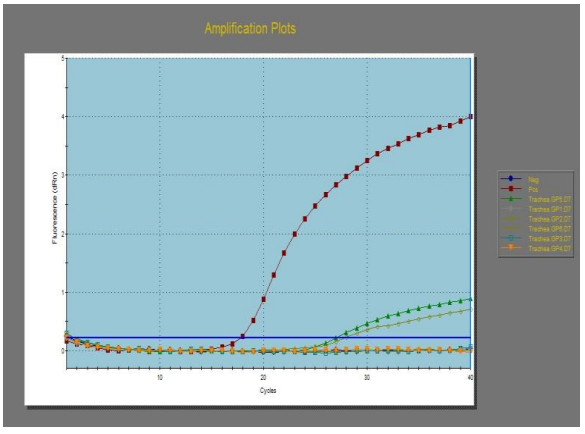


Fig (8): Real time -Polymerase chain reaction curve showing detection of IBV nucleic acid in oviduct at 7 days post vaccination

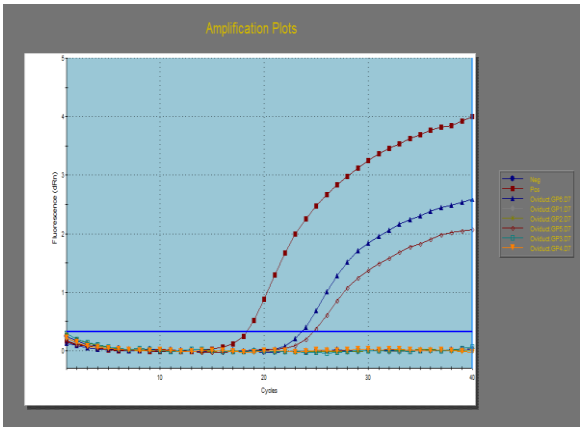
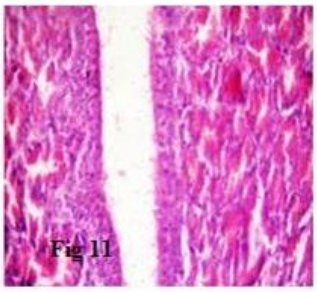


Fig (9):Real time -Polymerase chain reaction curve showing detection of IBV nucleic acid in oviduct at 14 days post vaccination



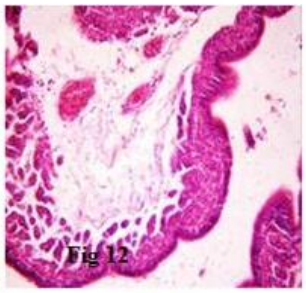


Fig.11: Oviduct of a hen from subgroup 6, showing loss of the cilia of luminal epithelium and showing lymphocytic aggregates in lamina propria. H&E stain x 200.

Fig. 12: Oviduct of a hen from subgroup5, showing congested blood vessels and marked edema in the lamina propria admixed with small numbers of mononuclear inflammatory cells, loss of the cilia of the luminal epithelium. H&E stain x 200.

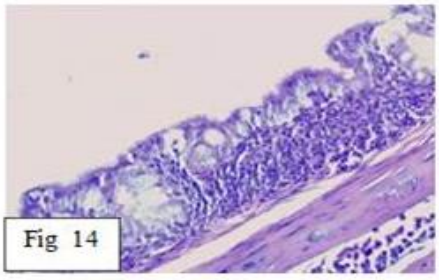


Fig 14: Trachea of G 4 showing hyperplasia of lining epithelium with activation of mucous glands (H&E X200).

Discussion:

IBV is an economically significant problem for the commercial egg industry globally despite the widespread use of live and inactivated vaccines. Loss of shell color in brown egg layers is a very common effect of IBV although it may also cause the production of thin shelled, misshapen, and corrugated eggs as well as more elongated eggs with loss albumen quality (Roberts *et al.*, 2017).

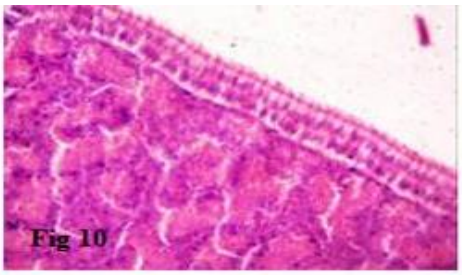


Fig.10: Oviduct of hen from subgroup (7) showing normal appearance of pseudostratified ciliated epithelium. The mucosal folds, glands, lamina propria, submucosa, tunica muscularis, and the serosa revealed normal histological criteria with H&E stain x 200.

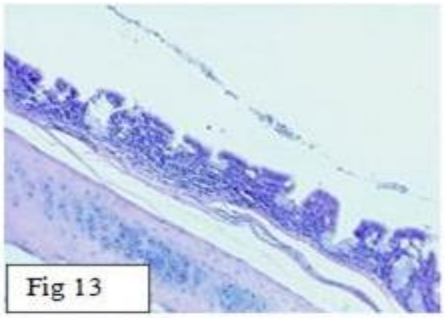


Fig 13 : Trachea of G 1 showing hyperplasia of lining epithelium with activation of mucous glands and mononuclear cells infiltration in lamina propria (H&E X200).

In this experimental study, we applied comparative study between prepared bivalent IBV inactivated vaccine and commercial one under field condition, also our attempt targeted for evaluation of both homologous and heterologous protection. In this study, we used four parameters as egg production, elevated antibody titers in serum and extracted egg yolk and protective titers in challenged chickens estimated by real-time reverse transcriptase-polymerase chain reaction (RRT-PCR) and histopathological examination for vaccine assessment.

A related study carried out by (Chousalkar *et al.*, 2009) who studied the effects of vaccine strains A3 and Vic S on the oviduct of laying hens and was assessed by histopathology, electron microscopy, serology and also by determining the presence of viral RNA in the oviduct by rRT-PCR following the experimental infection .

Our results come in comparison with (Mohamed *et al.*, 2018) who assessment the field efficacy of an attenuated IBV Variant 2 Vaccine in

commercial broiler chickens and his evaluated parameters depend up on assessment of humoral immunity with ELISA test and HI test , flock performance and viral shedding in tracheal and kidney samples with rRT-PCR and interference with other live attenuated against ND,AI and IBD.
The immune response was monitored by ELISA at weekly interval in the both vaccinated and control groups as shown in table (3) and figure (1) .After 5 weeks from vaccination group A showed elevated antibody titer followed by group B in comparison to control unvaccinated group. At the end of 1st week from challenge, heterologous challenge sub groups showed drop than homologous challenge one. As the same results in the second week challenge, there marked drop in heterologous challenge sub groups, it was obvious that heterologous challenge strain had marked effect on the mean antibody titer that was prominent in G4 than G2. Homologous and heterologous challenge sub groups (G5 and G6) recorded the highest level of antibody titer as a result of infection with IBV rather than vaccination, from analyzed data, vaccination with prepared bivalent IBV confer higher protective antibody titers against homologous and heterologous challenge than imported combined (ND&IB) as shown in table (4) and figure (2).
The recorded results can be discussed, that the mean antibody in the sera of vaccinated groups drop at the time of challenge due to neutralization effect for providing sufficient protection for the flock against infection as compared with control groups in which higher level of the mean antibody titer at the infection time.
Similarly, our results come in agreement with (Ghadakchi *et al.,* 2005) who observed that ELISA was used for the detection of antibodies against IBV. ELISA has been shown to be a reliable and sensitive method to monitor vaccination schedules and for the rapid detection of initial increase of antibodies against IB .
The formention results agree with (OIE,2018) which reported that regular monitoring of sera from flocks for IB antibody titers may help to indicate the level of vaccine or field challenge responses.
A related study carried out by (Ahmed *et al.,*2018) who showed that different vaccination programs using imported vaccines have minimal effect to protect the flocks from field challenge whereas a successful protection using homologous strains as live attenuated vaccines.
Egg production performance was estimated after vaccination ,control group showed increased in egg production in comparison to vaccinated groups .However ,the percent of egg production was numerically higher in group (A) in comparison to group (B). At 1st week post challenge, egg production showed obvious decrease in heterologous challenge subgroups than homologous challenge subgroups similar finding results in control challenge subgroups. At the end of 2nd week from challenge, heterologous challenge strain had progressive effect on the oviduct of control sub group G6 than sub group G5, on the other hand homologus challenge subgroups recorded gradual recovery in egg production than heterologous challenge sub groups . From recorded results, locally vaccine showed better effect on egg production even the time of challenge as shown in table (5),(6) and figure (3), (4).
Our findings came in agreement with that of (Muneer *et al.,*1986) who proved that the inactivated oil-emulsion IBV vaccines are commonly used to obtain long-lasting immunity to protect breeders and layers prior to the onset of the egg production.
The analyzed data from recording of egg production percentage could be higher in unvaccinated group than vaccinated one as more energy from bird nutrition directed for immune response rather than egg production that data come in agreement with (Cook *et al*., 1993) who illustrated that modulation of immune system by diet has been employed for two primary goals in poultry, to decrease the incidence of infectious diseases and to minimize the untoward effects of immune responses on growth, egg production and the incidence of metabolic diseases.
On the other hand, (European Pharmacopoeia, 2017) showed that the drop in egg production for Massachusetts types to be at least 35%, and for non-Massachusetts types to be at least 15% in non-vaccinated birds and statistical analyses should also be recommended in any vaccination-challenge study.
Similarly, our results come in accordance with (OIE, 2018) at which reported that inactivated

vaccines are often expected to protect against drops in egg production and vaccine complies with the test if egg production or quality is significantly better in the group having received the inactivated vaccine than in any control group.

In the vaccination –challenge study for assessment of both homologous and heterologous protection, IBV shedding was detected at 3rd dpi in the trachea of vaccinated and unvaccinated challenged subgroups, with higher virus titer in unvaccinated control sub groups. At 5 days post challenge, trachea from both G1 and G3 showed (–ve) reaction, while G2 and G4 still had lower viral titer than unvaccinated control sub groups. Birds in all vaccinated groups showed (-ve) reaction at 7 dpi and 14dpi but unvaccinated control sub groups had shedding (+ve) detection at 7 dpi in the oviduct samples. At 14 days post challenge, oviduct from G5 and G6 had higher viral titer than the first week from challenge as shown in table (7), (8) and figure (4), (5) , (6), (7) and (8).

Similarly, our finding results come in agreement with (Jackwood *et al.*, 2009; Benyeda *et al*., 2009; Jackwood and De Wit, 2013)who proved that the rRT-PCR assay is quick and highly sensitive technique which was up to 100 times more sensitive than virus isolation . Furthermore our observations agree with (Callison *et al*., 2006; Roh *et al*., 2014) who reported that (QRT-PCR) assays have been used to detect the presence of challenge virus when carried out experimental evaluation for the efficacy of IBV vaccine against field viruses.

The recorded results may be attributed to that vaccination with inactivated IB vaccine had good protection against homologous and heterologous challenge with decreasing viral shedding rate in vaccinated birds in parallel with unvaccinated one and this theory agree with the findings of (De Jong and Diekmann, 1991;De wit and Cook 2014) who define the protection with simple terms as the ability of an IBV vaccine to prevent infection of the chicken following challenge with a field strain of IBV.

Recently protection studies of live attenuated variant strains indicated that using local strain vaccine can provide better protection against IBV challenge (Lee *et al*., 2010; Zhao *et al*., 2015), also, attempts to widen the protective efficacy of prepared IBV vaccines through experimental study of combined live IBV vaccines showed successful strategy to protect chicken against heterologous virulent IBV strains (Gelb *et al* ; 2005).

Similarly, our results come in agreement with (Walaa, 2016) who evaluated the efficacy of combined inactivated (ND&IB) vaccine in layer chickens against nephropathogenic variant II genotype (IS/1494) IBV and she found that all vaccinated groups showed (-ve) reaction by using rRt-PCR at 10 days and 14 days post challenge which indicated that the inactivated vaccine gave protection in oviducts.

A related study carried out by (Ahmed *et al.*, 2018) who assessed the efficacy of live attenuated classical and variant 2 IBV vaccine by rRT-PCR and his results revealed that the prepared vaccine was able to reduce the shedding of the challenge at 3 days post-infection (DPI) and no virus shedding was detected in vaccinated group by 5 DPI with homologous challenge strain.

In the present study the histopathological findings of trachea and oviduct samples revealed presence of severe microscopic lesions in the control challenge subgroups than vaccinated challenges ones as showed in the recorded figures (10,11,12,13,14 and15).

Our results come in agreement with (Riddell , 1987; Chausalkar and Roberts , 2007) who showed that tracheal mucosa of chickens infected with IB is edematous, there is a loss of cilia, rounding and sloughing of epithelial cells, and minor infiltration of lymphocytes, other mononuclear cells, plasma cells, heterophils and lymphocytes, experimental IBV infection of oviduct in mature hens resulted in dilation of the tubular glands and edema of the oviduct mucosa.

5. CONCLUSION

This study indicated the field efficacy of the newly developed bivalent inactivated IBV vaccine in commercial layer chickens. Moreover, it appears that the combination of classical and variant II inactivated IBV vaccines confers a protective immune response and protect drop of egg production against both homologous and heterologous viral challenges.

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