Comparative study between prepared bivalent IB inactivated vaccine and commercial one

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ABSTRACT:

Infectious bronchitis is a highly contagious disease and considered as one of the most important and common poultry diseases and it causes huge economic losses in poultry industry due to its effect on broiler growth and its effect on egg production quantity and quality in adult hens. Inactivated vaccines are important component in vaccination program for layer and breeder for protection against egg production and also provide early protection against infection through maternal immunity in progeny. This study was conducted to compare the efficacy performance between prepared bivalent IB inactivated vaccine and commercial one in commercial layer chickens, vaccinated chickens from both groups were challenged for evaluation of both homologous and heterologous protection, and all these steps were compared with control non vaccinated groups. Enzyme linked immunosorbent assay (ELISA) was used for estimation of humoral immunity and also transmissible maternal immunity through laid egg yolk post vaccination from all groups, challenge strains were detected by using Real-Time RT-PCR from tracheal and oviduct samples in all groups and also histopathological changes were evaluated after challenge test from collected samples. The results revealed that the prepared vaccine give higher protective antibody titer in both serum and egg yolk as commercial vaccine which provided good protection against both homologous and heterologous challenge with comparison in control groups. Post challenge changes in trachea and oviduct samples take good evaluation in vaccinated groups with histopathological techniques. This confirms that under field condition,

poultry industry can be protected from IB disease if using locally prepared inactivated vaccine as look like imported commercial one in Egyptian layer farms.

Key words: Infectious bronchitis virus, layer chickens, inactivated vaccine

Introduction:

The 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 result in nephritis .In adult, IBV effect on reproductive tract and produce irreparable damage to oviduct and production of abnormal eggs. (**Arthur Sylvester *et al*., 2005; Cook *et al*., 2012; Jackwood, 2012; Jackwood and de Wit, 2013**).

IBV is a gammacoronavirus, family coronavirdae, order Nidovirales (**Cavanagh and Naqi, 2003**). The virus is a single-stranded, positive sense, 27 kb RNA genome that encodes many nonstructural proteins involved in replication, three major structural proteins (spike (S), envelope (E), membrane glycoprotein (M) involved in virion formation and a protein involved in genome packaging nucleocapsid (N) (**Cavanagh, 2007**). The S glycoprotein is cleaved into two subunits, S1 and S2, of these proteins, S1 is often regarded as the most significant due to its role in host cell binding and neutralizing epitope presentation (**Promkuntod *et al*., 2014**).

IBV has a constant threat to the poultry industry although vaccination has been considered to be the most cost effective approach to controlling IBV infection (**Meeusen *et al*; 2007**). Even though the poultry industry extensively vaccinates against IBV, Immunization programs now seem to be ineffective due to the extreme genetic variations of IBV. Inactivated IB vaccines produced high and uniform levels of maternal

antibody which persist for longer periods in comparison to broilers from dams vaccinated with live vaccines only. Moreover inactivated vaccines do not replicate, they are unlikely to revert and cause pathological effects **(Gough and Alexander, 1978**; **Ignjatovic and Sapats., 2000).** Both live and attenuated vaccines are used in IBV immunization programs in broiler and breeders. Inactivated vaccines provide protection against drop in egg production, which may not always be given by live vaccination (**Box *et al*, 1980**). Importantly, some IBV strains to which vaccines become available might disappear as new variants emerged and thus necessitate the development of new vaccines (**Meeusen *et al*; 2007**). The aim of the present study was directed to comparative study between locally prepared bivalent IB inactivated vaccine and commercial combined (IB & ND) inactivated vaccine and also evaluation of transmissible maternal immunity through laid egg yolk.

2. MATERIALS AND METHODS:

2.1. Experimental host:

Two hundred and thirty chickens aged, 17 week old Red Lohman chicken 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 Infectious Bronchitis disease, the vaccine commonly used was the live vaccine based on eye drop (hitchner IB H120) at 7 day of age and inactivated (IB & ND) vaccine at 30 day of age.

Table (1): Vaccination program applied during the rearing of layer chickens

Age of bird / day	Type of vaccine	Vaccination route
1st	Markes disease vaccine	S/C injection in hatchery
7th	IB (H120) + ND (HB1)	Eye drop
10th	AI (H5N1)	S/C injection
13th	IBD (first dose)	Eye drop
17th	ND LaSota	DW
21st	IBD (second dose)	Eye drop
30	IB (M41) + ND (colon 30)	I/M injection
38th	ILT	Eye drop
55th	Pox	Stamping in wing web
70th	AI (H9N2)	S/C injection
85th	Inactivated coryza and cholera	I/M injection
95th	ND LaSota	DW

2.2. IB vaccines:

1- Prepared bivalent inactivated IB vaccine containing (classical strain KP279995/2014 and variant strain KP279998/2014) (**Reda *et al*; 2019**).

2- Commercial combined inactivated vaccine contain 500 ml (1000 doses) of the following virus strain (Infectious bronchitis IB M41& Newcastle disease Clone 30). Batch number (59024), purchased by Intervet International, Holland

2.3. Infectious bronchitis challenge strains:

2.3.1. Homologous challenge strains:

Strain of IBV (Chicken / Egypt/ KP279995 / VACSERA / 2014, Classical) and (Chicken / Egypt KP279998 / VACSERA / 2014, variant) **(Reda *et al*; 2015)** with infectivity titer of $10^{4.5}$ EID_{50} /ml.

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 with infectivity titer $10^{5.8}$ EID_{50} /ml.

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

ELISA Kit was obtained from Biocheck poultry immune assays. Infectious Bronchitis Virus antibody test kit (CK119): Serial No. F69371 product code: 6020. For estimation of humoral immunity and also transmissible maternal through laid egg yolk for IBV after vaccination. All procedures were conducted according to manufacturer's instructions.

2.5. Real-time Reverse transcriptase chain reaction (RRT-PCR):

Tracheal and oviduct samples were collected and tested for detection and titration of IBV in both vaccinated and control groups at 3,5,7, 14 days post challenge 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) provide accurate real-time quantification of RNA targets, Primers and probes used were supplied from Metabion (Germany) as shown in table (2). With the following cycling conditions: reverse transcription at 50°C for 30 min , primary denaturation at 95°C for 15 min; 40 cycles of 94°C for 15 sec; 60°C for 45 sec (annealing +extension).The numbers of viral genome copies were quantified in a TaqMan® real time RT-PCR targeting IBV gene.

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

2.6. Chemical reagents and solutions:

2.6. A.Phosphate buffer saline (PBS):

Constitutes were diluted in 2 liters of D.W, PH adjusted to 7.2, PBS was used as diluents for virus suspension, preservation of samples and used in egg yolk extraction. Antibiotic solutions as streptomycin and penicillin –G sodium was added to each 100 ml of buffer solution.

2.6. B. Chloroform:

Pure reagent grade chloroform was purchased from El-Nasr pharmaceutical Chemicals. Co. Cairo, Egypt, and used for egg yolk extraction.

2.7. Reagents for histopathology according to (**Bancroft and Steven, 1977**) it was carried for recording changes in trachea and oviduct.

2.8. 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 ($P<0.05$).

Experimental design:

Two hundred and thirty Red Lohman layer chicken aged 17-week-old were divided into 3 groups; group A and B contain 100 chickens for each, while group C contain 30 chickens. At the end of 18 week old the

divided groups were treated with different manner as first group (A) vaccinated with 0.5 ml S/C per bird of locally prepared bivalent IB inactivated vaccine, second group (B) vaccinated with commercial combined IB &ND inactivated vaccine and third group (C) were kept as unvaccinated control group. Blood samples were taken weekly after vaccination till the end of experiment for evaluation of humoral immunity from all groups, also estimation was continued in vaccinated groups till 6 months for follow-up constituting immunity. Egg performance parameter were evaluated in the three groups till the end of experiment, egg yolk was collected at 3^{th} , 4^{th} and 5^{th} week post vaccination for evaluation of transmitted material immunity.

Challenge test:

After one month from vaccination, groups A and B were divided into 2 subgroups; while group C were divided into 3 subgroups. 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 for estimation of both homologous and heterologous protection as shown into table (3). Monitoring of viral shedding from both vaccinated and unvaccinated control groups by collection of tracheal swabs at interval 3^{th}, 5^{th} and 7^{th} days post challenge, oviduct samples at interval 7^{th} , 10^{th} and 14^{th} days post challenge, also histopathological changes were recorded in different groups.

Table (3): Lohman layer chickens were challenged at 22 week old by homologous and heterologous strains.

Group	No of birds/group	Treatment	Challenge
A	50 50	prepared vaccine	Group 1 ⟶ Homologous challenge Group 2 ⟶ Heterologous challenge
B	50 50	commercial combined (IB&ND) vaccine	Group 3 ⟶ Homologous challenge Group 4 ⟶ Heterologous challenge
C	10 10 10	Unvaccinated control	Group 5 ⟶ Homologous challenge Group 6 ⟶ Heterologous challenge Group 7 ⟶ control (-)

Yolk extraction:

laid eggs were collected after 2 weeks post vaccination from both vaccinated and unvaccinated control groups, yolk of egg weighted 50 gm was separated from the white .Needle was used to dispense 1 ml of the yolk to a centrifuge tubes and 1 ml of phosphate buffer saline (pH 7.2) to make a dilution of 1:1 (vol/vol), two ml of pure grade chloroform reagent was added to a test tube containing 1ml of the 1:2 diluted egg yolk suspension, left at room temperature for one hour and tubes were centrifuged at 3000 rpm for 20 minutes. The upper layer was removed then the solution was dispensed in Eppendorf tubes and stored at – 20 C until used for testing according to method described by (**Mohammed *et al*, 1986).** Using of ELISA manual protocol for yolk extracted samples as evaluated parameter for transmitted antibody titer through yolk via higher serum level

3. Results:

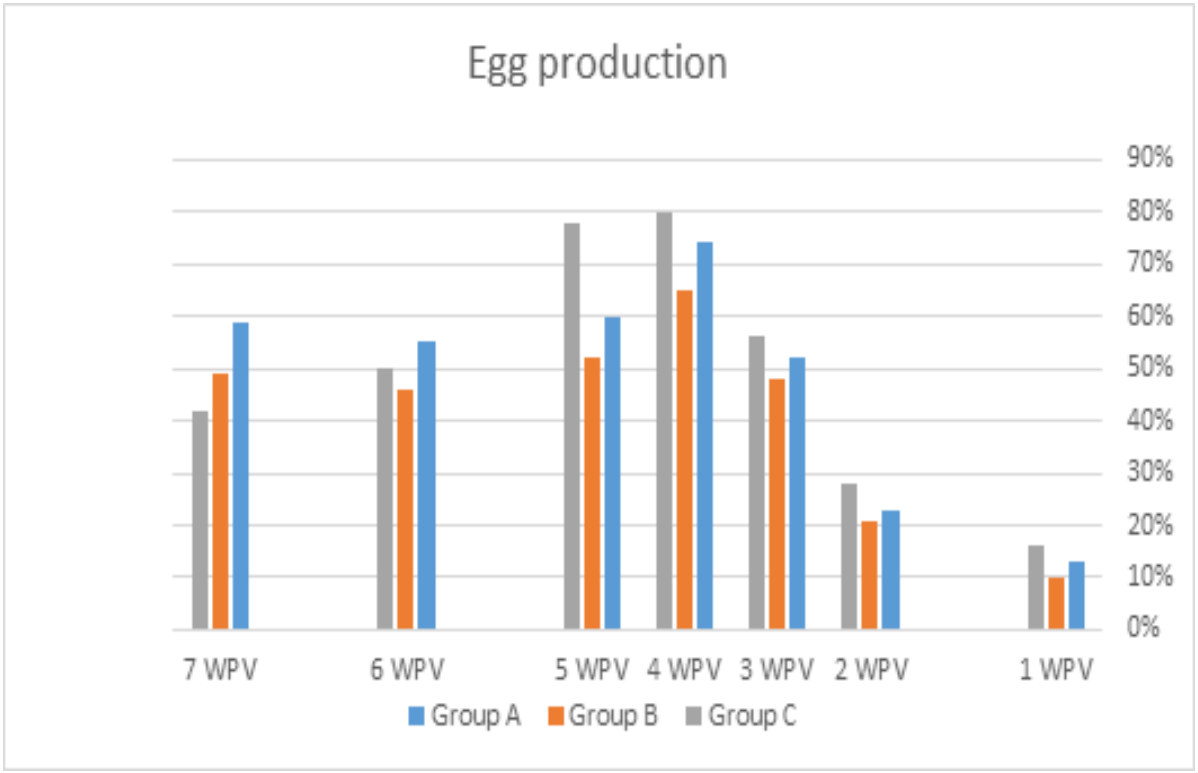
1-Egg performance parameter after vaccination and challenge in three groups:

Three groups evaluated for egg production after vaccination and challenge, (vaccinated groups (A and B) and control group (C). Vaccination was carried at the end of 18 week old and after one month from vaccination at 23 week old, challenge with IBV occurred. The percent of egg production was significant increase in the group (A) when comparison with group (B) before and after challenge. In the control group (C) had significant decrease in egg production percent at 6 week and 7 week from vaccination time in which challenge was carried out as in table (4) and figure (1).

Table (4): Egg production percentage in 3 groups per week

Group/week	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 %
Six week post vaccination 1st week challenge	55 %	46 %	50 %
Seven week post vaccination 2nd week challenge	59 %	49 %	42 %

Figure (1): Effect of IBV challenge in both vaccinated (A, B) and unvaccinated control group (C) on egg production.



2-Results of ELISA of IB:

At first 3 weeks after vaccination, there was mild numerical difference in titers as illustrated in table (5) and figure (2).However there was higher titer in the first group in comparison with group 2 and group 3. At 4th week, the vaccinated groups recorded significant (P<0.05) increase in comparison to control (–ve) group, while there was non-significant (P>0.05) changes between first and second group. First group showed higher ELISA titer at 5th week from vaccination (

Table (5): The Mean antibody titer to both vaccinated groups and also control before and after challenge using ELISA test.

Group/ weeks	G1	G2	G3
Before vaccination	855.27±637. 9^{a}	855.27±637.9^{a}	8552.7±637.9^{a}
2 week post vaccination	7912.0±187.2^{a}	7303.7±160.9^{a}	7036.0±103.2^{a}

3 week post vaccination	843.4±217.1[a]	750.6±187.1[a]	569.8±139.2[a]
4 week post vaccination	1030.6±83.07[a]	1202.8±74.14[a]	475.80±159.9[b]
5 week post vaccination	1260.0±36.7[a]	1002.6±12.2[b]	244.3±35.4[c]
6 week post vaccination	980.0±105.6[a]	951.6±117.4[a]	933.4±91.84[a]
7 week post vaccination	884.27±130.1[a]	830.67±71.41[a]	1006.9±71.12[a]

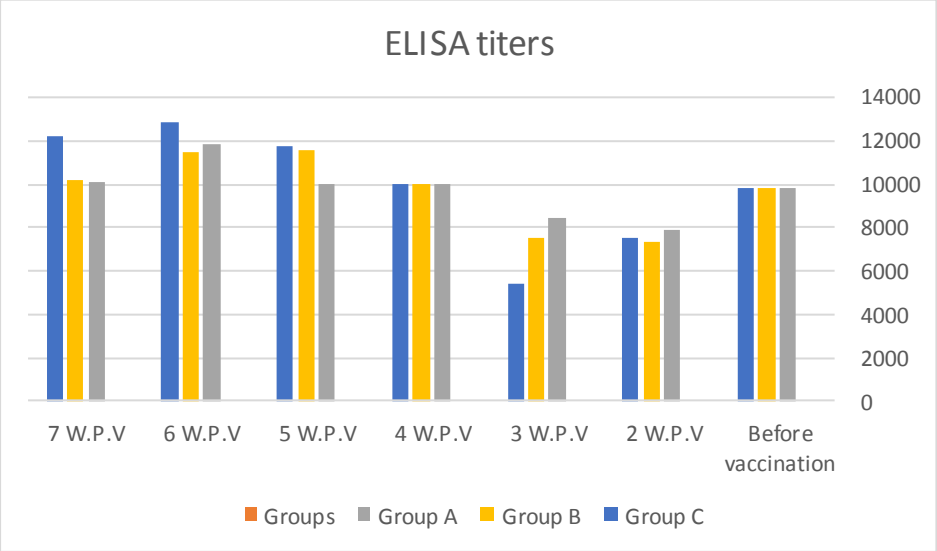


Fig (2) The antibody response to locally prepared IB inactivated vaccine and commercial combined inactivated IB +ND vaccine with comparison in control unvaccinated group before and after challenge using ELISA test in commercial layers.

D.P.V: Days post vaccination

N.B: challenge at 35 days from vaccination

Group A: vaccinated with locally prepared IB inactivated vaccine.

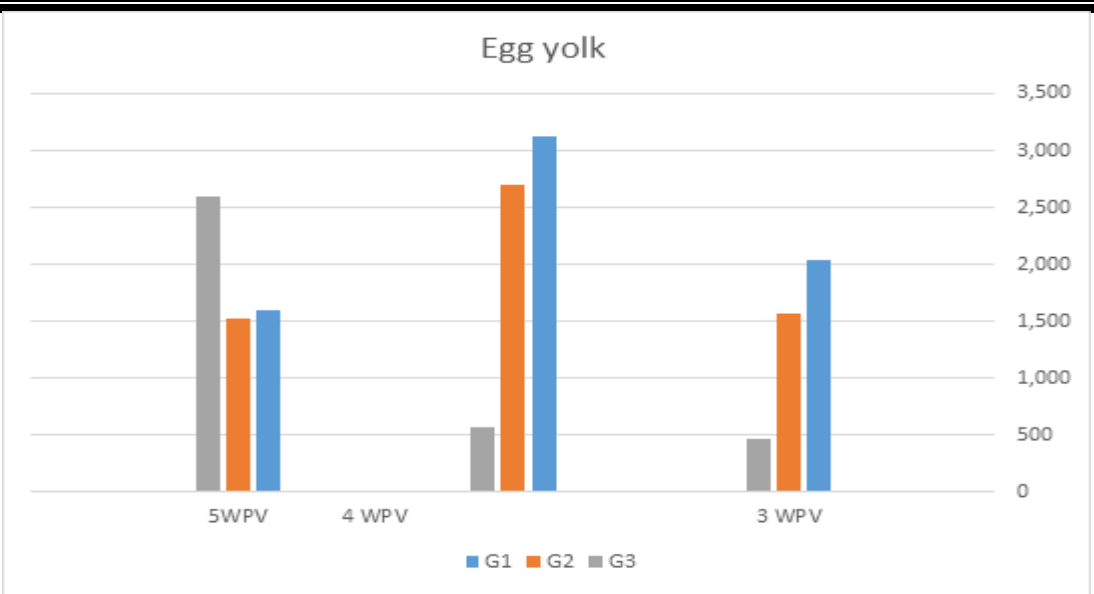
Group B: vaccinated with commercial combined inactivated IB +ND vaccine

Group C: unvaccinated control

3-Egg yolk serology

As in table (6) and figure (3)

	GA	GB	GC
3 WPV	2,039	1,571	458
4 WPV	3,129	2,699	373
5WPV	2,591	1,522	232



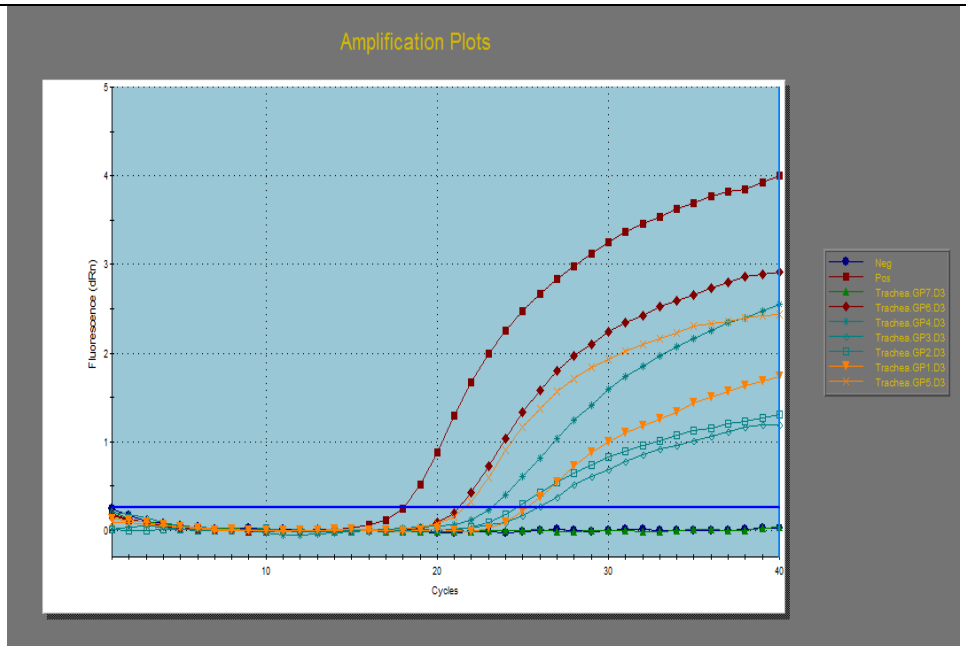
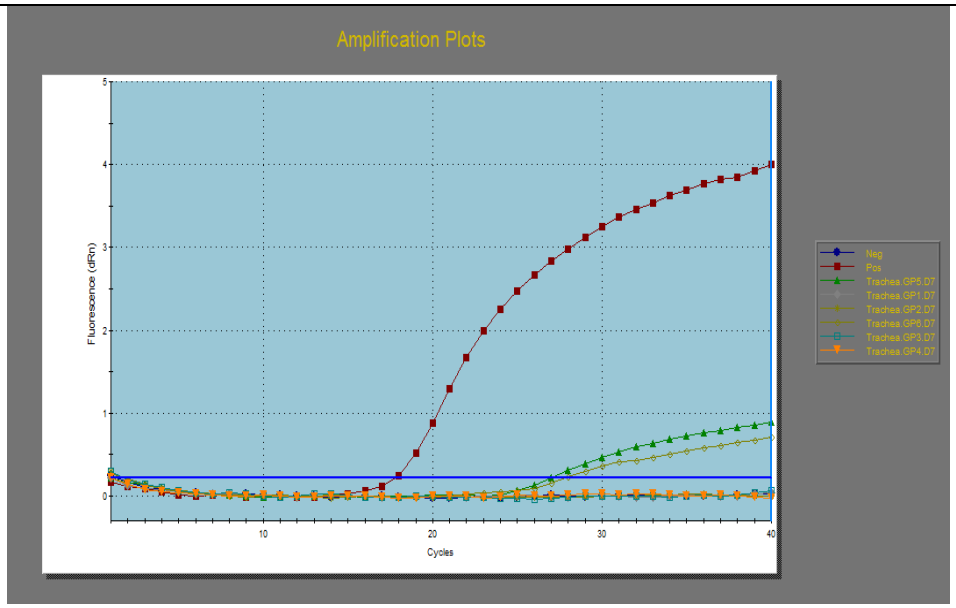
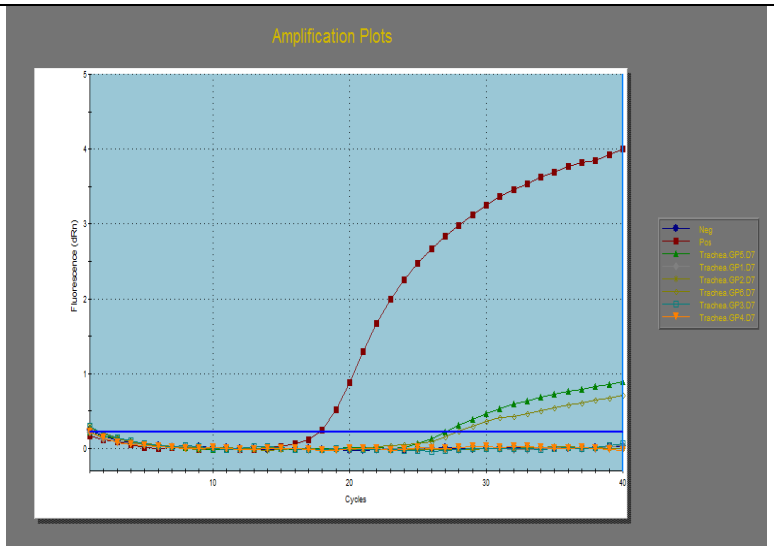
4-Detection of IBV nucleic acid in vaccinated and challenged layer chickens evaluation of shedding test for locally prepared bivalent inactivated IB vaccine after challenge in both trachea and oviduct samples:

Real-time RT-PCR has been used for successful detection of IBV nucleic acid in vaccinated birds and unvaccinated birds. Nucleic acid of IBV could first be detected at 3 days post challenge only in the trachea of vaccinated and unvaccinated challenged groups, with higher virus titer in G5 and G6 unvaccinated control groups. At 5 days post challenge, trachea

from both G1 and G3 with homologous challenge were (–ve) reaction, while still vaccinated group as G2 and G4 with heterologous challenge had lower viral titer than control (+) G5 and G6. Birds in all vaccinated groups were (-ve) reaction at 7 days post challenge with comparison with (+) reaction with unvaccinated control group G5 and G6. Vaccination with inactivated IB vaccine had good protection against homologous and also heterologous challenge with decreasing viral shedding rate in vaccinated birds in parallel with unvaccinated one as shown in table (7) and figure (4), (5) and (6).

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

Sample type	Group	Day 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	-	-

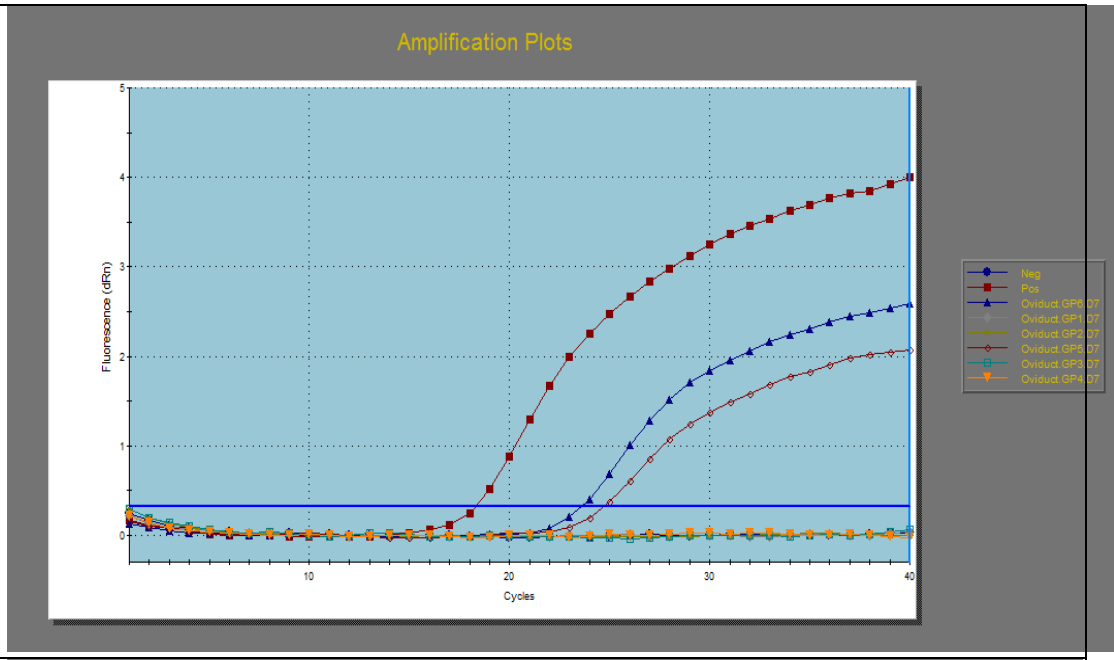
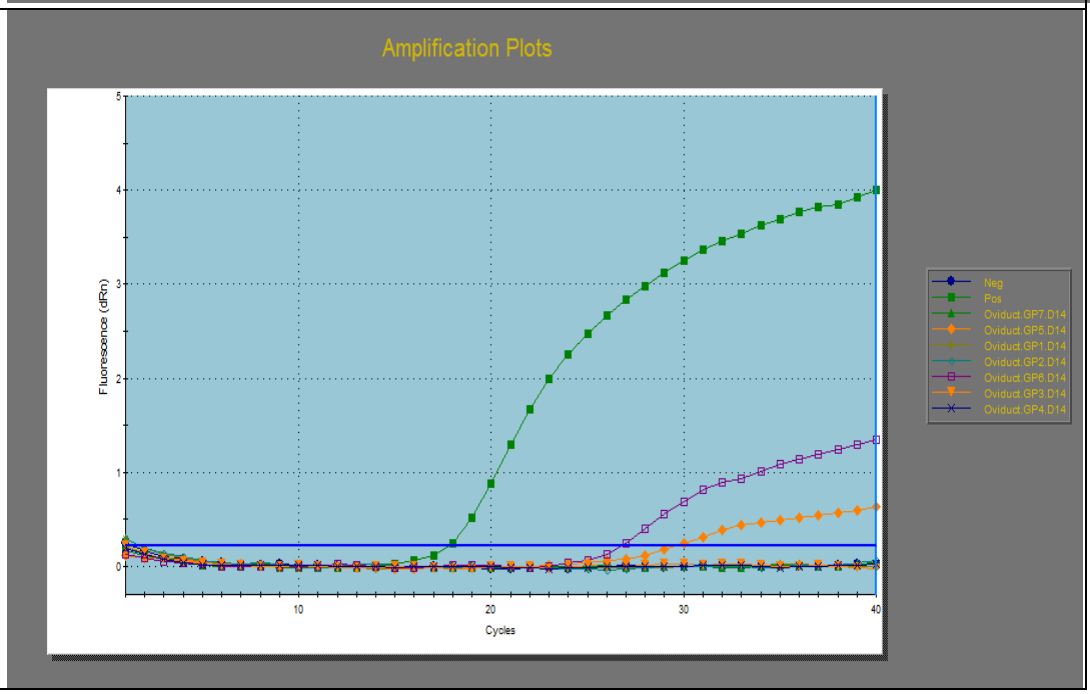
Fig (4):Real time – PCR Curve showing detection of IBV nucleic acid in trachea at 3 days post vaccination	
Fig (5):Real time – PCR Curve showing detection of IBV nucleic acid in trachea at 5 days post vaccination	
Fig (6):Real time – PCR Curve showing detection of IBV nucleic acid in trachea at 7 days post vaccination	

2-Oviduct samples:

Real-time RT-PCR has been used for successful detection of IBV nucleic acid in vaccinated birds and unvaccinated one. Nucleic acid of IBV could first be detected at 7 days post challenge only in the oviduct of unvaccinated challenged groups only. At 14 days post challenge, oviduct from both G5 and G6 had higher viral titer than the first week from challenge, birds in all vaccinated groups (G1,G2,G3,G4) had (-ve) reaction in two times of nucleic acid viral detection . That mean with inactivated IB vaccination give good protection against homologous and also heterologous challenge in comparison with unvaccinated one as shown in table (8) and figure (7) and (8).

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

Sample type	Group	Day 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 (7):Real time –PCR Curve showing detection of IBV nucleic acid in oviduct at 7 days post vaccination	
Fig (8):Real time –PCR Curve showing detection of IBV nucleic acid in oviduct at 14 days post vaccination	

5-Histopathological examination

Post –mortem examination finding after challenge with the help of staining and microscopical examination in vaccinated (G1, G2, G3, and G4) and unvaccinated control (ve+) group (G5, G6) with comparison with control (ve-) group (G7).

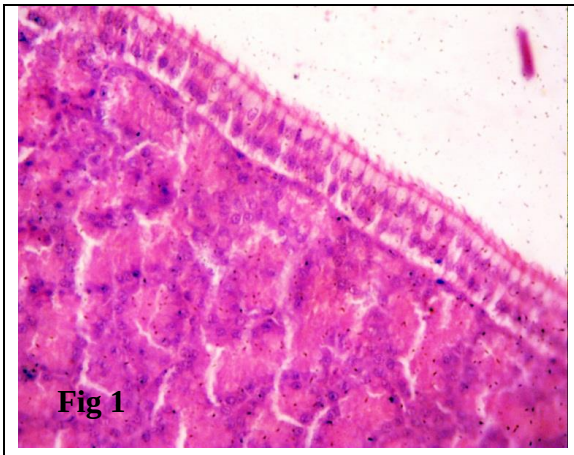
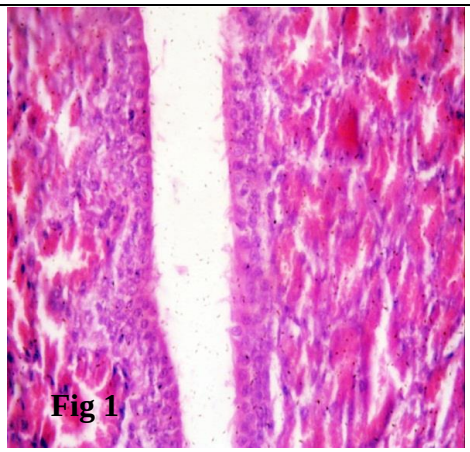


Fig.1: Oviduct of hen from group (7) showing normal appearance of pseudostratified ciliated epithelium. The mucosal folds, glands, lamina propria, submucosa, tunica muscularis, and the serosa revealed normal histological criteria with H&E stain x 400.



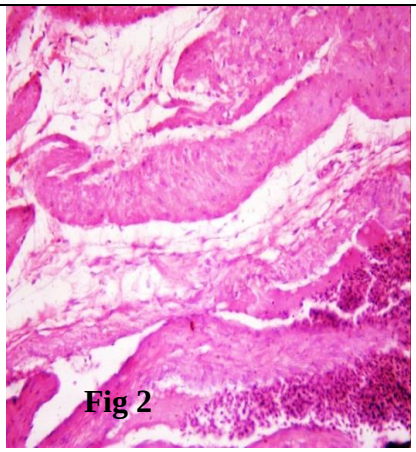


Fig.1: Oviduct of a hen from group 6, showing loss of the cilia of luminal epithelium and showing lymphocytic aggregates in lamina propria. H&E stain x 200.

Fig.2: showing congested blood vessels and intermuscular edema admixed with few lymphocytes in tunica muscularis. H&E stain x 400.

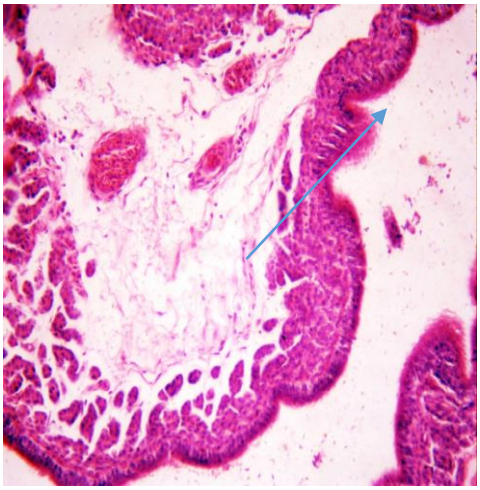
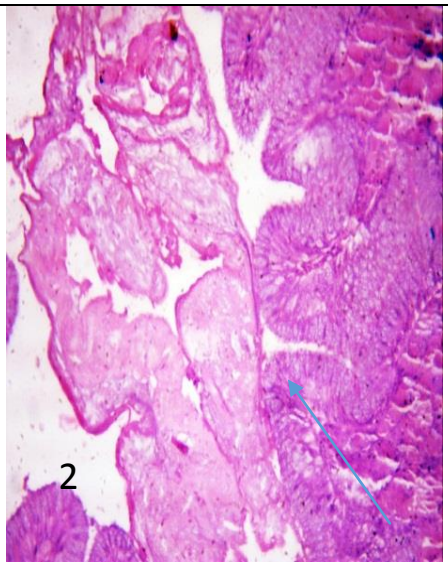


Fig. 1: Oviduct of a hen from group 5, showing congested blood vessels and marked edema in the lamina propria admixed with small numbers of mononuclear inflammatory cells, loss of the cilia of the luminal epithelium. H&E stain x 200.



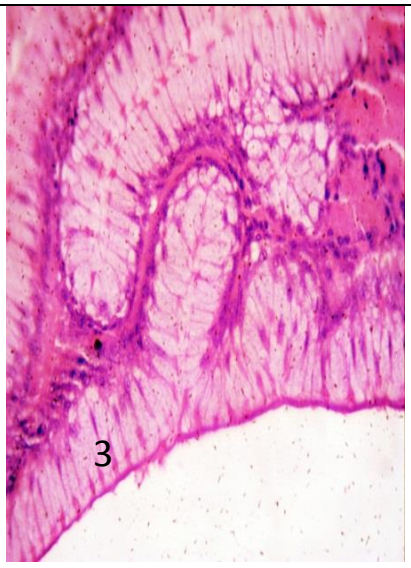


Fig. 2: Oviduct of a hen from group 5showing excessive mucus exudates on the mucosa. H&E stain x 200.

Fig. 3: Oviduct of a hen from group 5 showing marked hyperplasia and mucous metaplasia of luminal epithelium. H&E stain x 400.

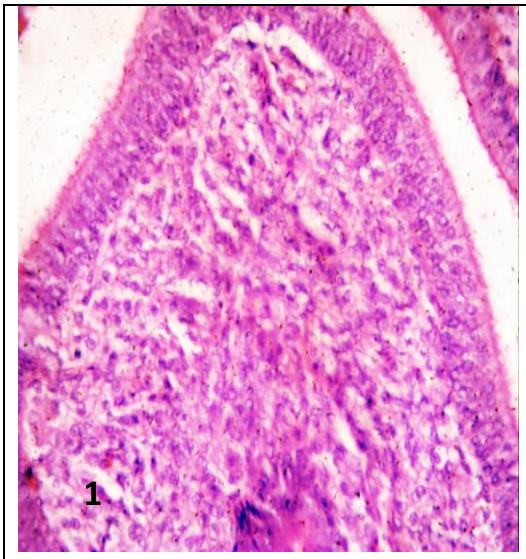


Fig. 1: Oviduct of a hen from group 2 showing mild vacuolar and hydropic degeneration of glandular epithelial cells characterized by swollen pale vacuolated cytoplasm were observed in the uterine mucosa The different portions of oviducts showed normal histological criteria.H&E stain x 400.

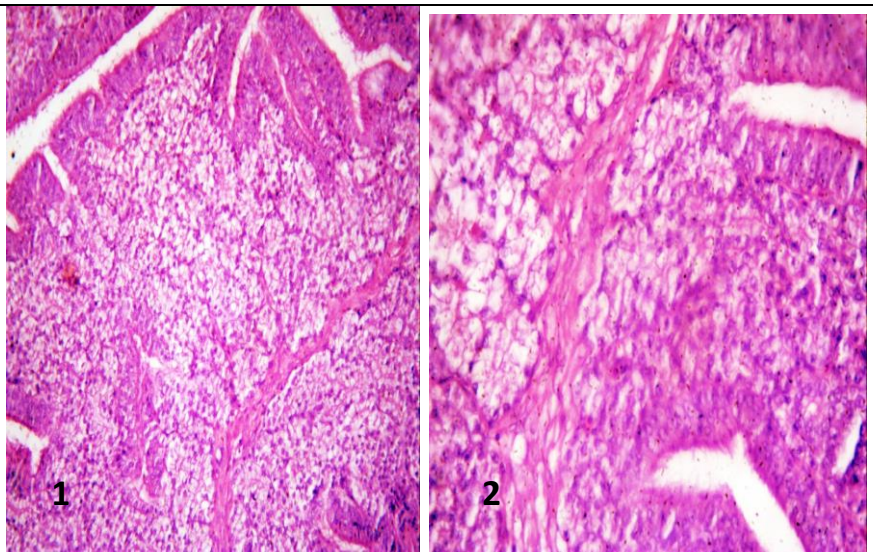


Fig. 1: Oviduct of a hen from group 1 showing multifocal to coalescent areas of hydropic degeneration of glandular epithelial cells.H&E stain x 200.

Fig. 2: Oviduct of a hen from group 1 showing hydropic degeneration of glandular epithelial cells characterized by large pale vacuolated cytoplasm .H&E stain x 400

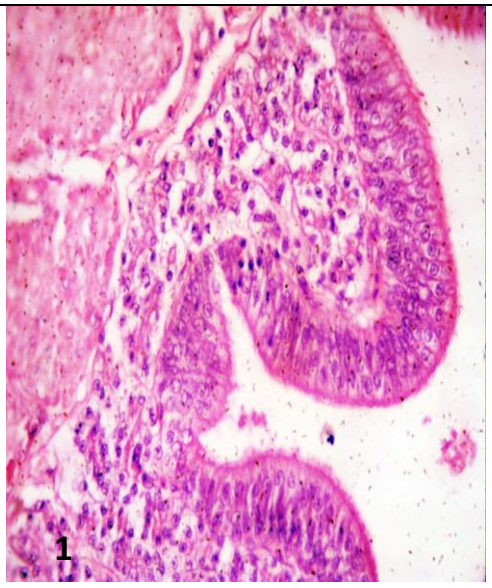


Fig. 1: Oviduct of a hen from group 3 showing mild histopathological changes in the uterus. Glandular cells of the uterine mucosa revealed vacuolar and hydropic degeneration characterized by swollen pale vacuolated cytoplasm H&E stain x 400.

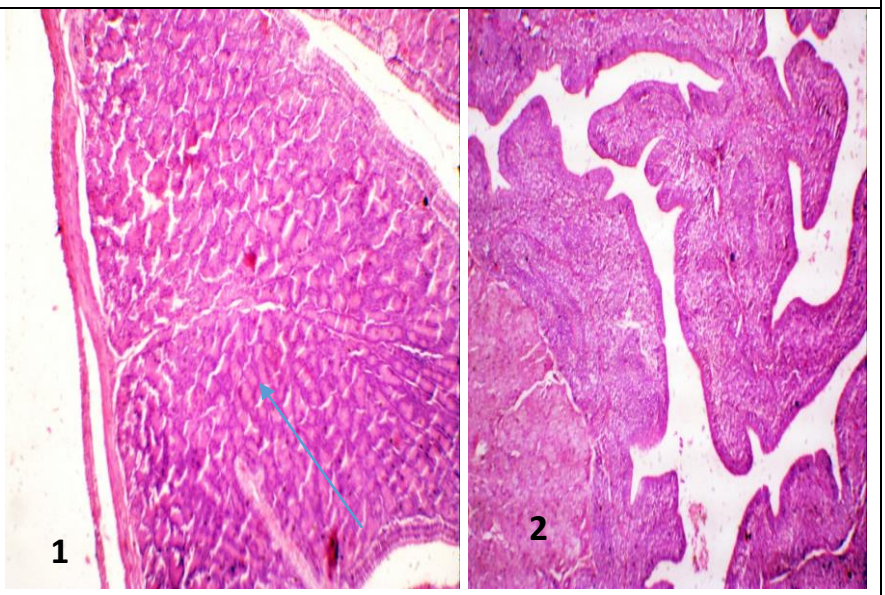


Fig. 1: Oviduct of a hen from group 4 showing normal histological appearance of pseudostratified ciliated epithelium, mucosal glands and tunica muscularis of the magnum. H&E stain x 100.

Fig. 2: Oviduct of a hen from group 4 showing normal histological criteria of mucosal folds, glands, and muscular wall of the uterus. H&E stain x 200.

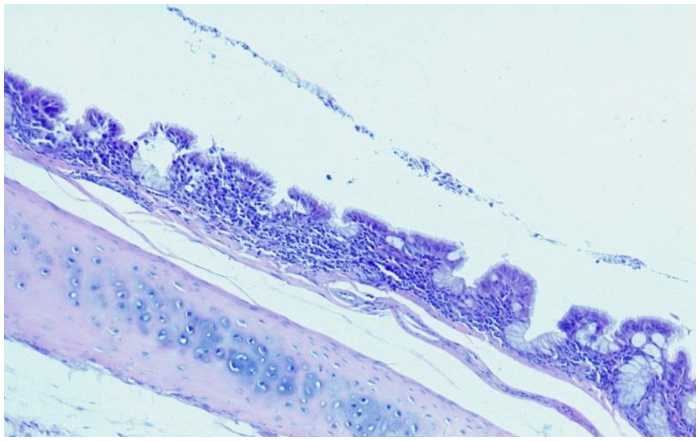


Fig. 1 Trachea of G 1 showing hyperplasia of lining epithelium with activation of mucous glands and mononuclear cells infiltration in lamina propria (H&E X200).

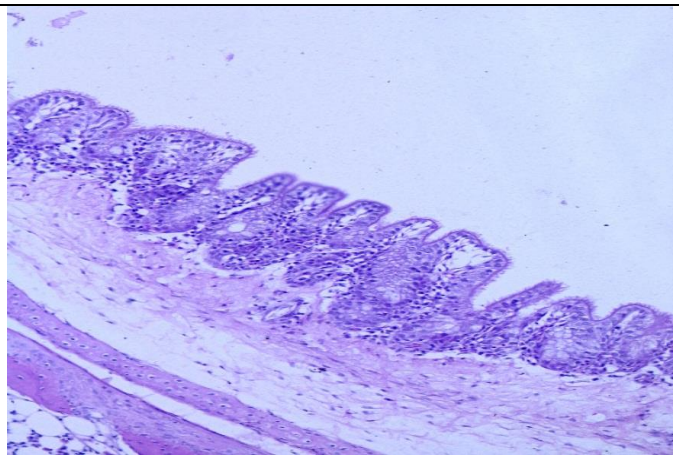


Fig 1: Trachea of G 2 showing focal hyperplasia of the lining epithelium with few mononuclear cells infiltration in lamina propria and submucosal edema (H&E X200).

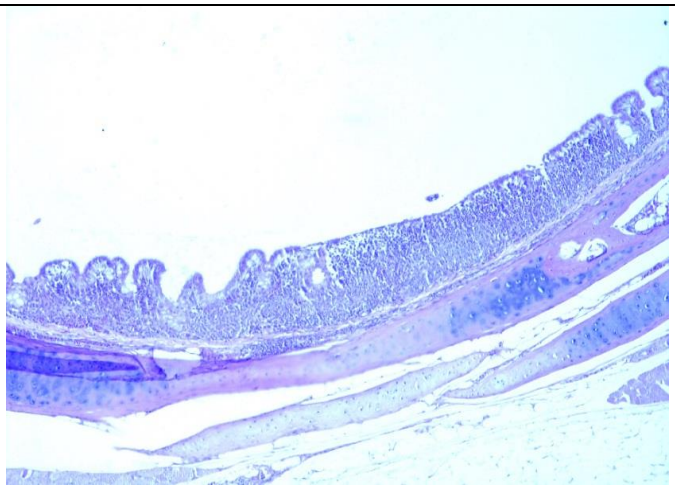


Fig 1 :Trachea of G 3 showing hyperplasia of lining epithelium with activation of mucous glands and thickening of mucosal layer with mononuclear cells infiltration in lamina propria (H&E X200).

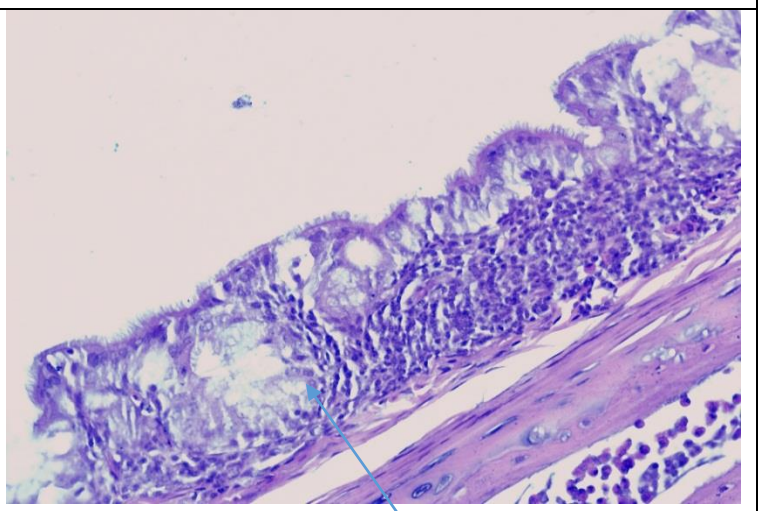


Fig 1: Trachea of G 4 showing hyperplasia of lining epithelium with activation of mucous glands (H&E X400).

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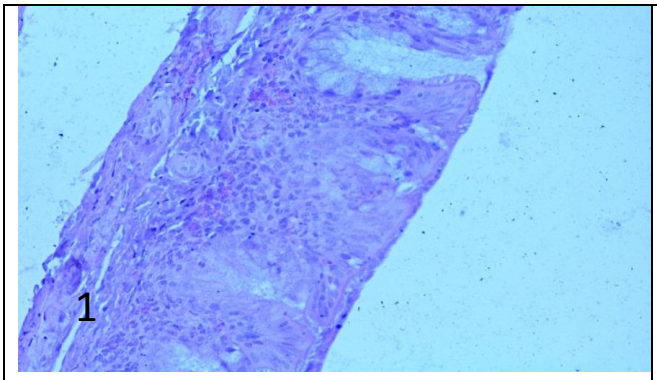
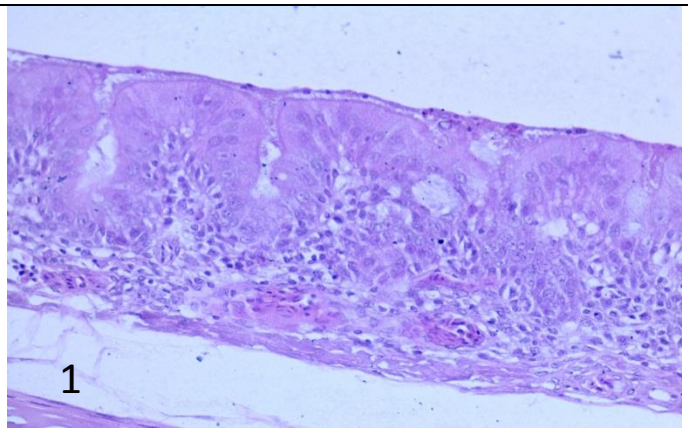
<tr><td> 
</td><td> 
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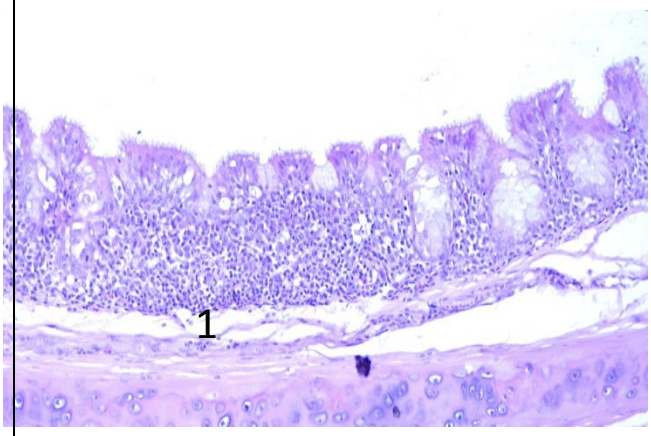
<tr><td>Fig 1:Trachea of G 5 showing thickening of mucosal layer with few granulocytes infiltration in lamina propria (H&E X400)</td><td>Fig 1: Trachea of G 6 showing hyperplasia of lining epithelium with activation of mucous glands and congested blood vessels (H&E X400).</td></tr>
<tr><td> 
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<tr><td>Fig 1: Trachea of G 7 showing focal thickening of mucosal layer with mononuclear cells infiltration (H&E X200).</td><td></td></tr>
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4. Discussion:

Infectious bronchitis virus (IBV) is an economically significant problem for the commercial egg industry globally despite the widespread use of live and inactivated vaccines. The presence of multiple serotypes and the rapid evolution of this virus complicate prevention of the disease by vaccination. Many serotypes cause drops in egg production accompanied by reduced shell and albumen quality. 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. The IBV produces pathological effects on body tissues including the oviduct, which can be clearly identified by light and electron microscopy **(Juliet and Kapil, 2017)**.Outbreaks of infectious bronchitis have declined in recent years as a result of the extensive use of vaccines; however, the disease may occur even in vaccinated flocks when immunity is waning, or upon exposure to variant virus serotypes, with the first variant strains emerging in the 1940s and new variants continue to emerge today. To minimize this risk, most poultry producers obtain 1-day-old chicks from maternal antibody-positive breeders and then spray-vaccinate them with live-attenuated vaccine in the hatchery, with additional boosts by live-attenuated and/or inactivated vaccines. The first vaccination is typically given in the hatchery when birds are 1 day old, and booster vaccination is given at 10–18 days. Passively acquired maternal immunity prevents respiratory infection and disease for the first 7 days. For layers or breeders, live-attenuated vaccines are used for priming, followed by killed oil-adjuvanted booster vaccines, often given repeatedly during the laying cycle. Vaccination breaks occur because of the variable presence of new antigenic variants and existence of several serotypes. Such variants will continue to emerge and spread, posing continuing problems for poultry producers. Control of infectious bronchitis is difficult because of the presence of persistently infected variant viruses. The present study was an attempt for comparative study between locally prepared bivalent IB inactivated vaccine and commercial one under field condition which coincides with the best time for using in vaccination programme for layer chickens. That study targeted for evaluation of both homologous and heterologous protection in both vaccinated groups with comparison in control unvaccinated one**,** the parameters were conducted on egg production, elevated antibody titers in serum and extracted egg yolk and protective titers in challenged chickens estimated by Real-time PCR and histopathological examination. The results of egg performance in vaccinated and control unvaccinated chickens

In the present trial, the effects of vaccine strains A3 and Vic S on the oviduct of laying hens were assessed by histopathology, electron microscopy, serology and also by determining the presence and persistence of viral RNA in the oviduct by real time PCR following the experimental infection In Australia, currently, all pullets reared for egg production are vaccinated with live attenuated strains of infectious bronchitis virus. Various vaccines and protocols to control this viral disease have been developed, although the severity of the

disease varies from place to place and flock to flock. **(Chousalkar *et al*;2009)**

ELISA in serum egg

Real-time PCR

Histopathology

Conclusion:

References:

Juliet, R.Roberts and Kapil, K.Chousalkar (2017): Egg Innovations and Strategies for Improvements, Chapter 52 - Infectious Bronchitis.561-570.

Chousalkar.,K.K; Cheetham B.F.;Roberts; J.R. (2009) : *Effects of infectious bronchitis virus vaccine on the oviduct of hens.* Vaccine Volume 27, Issue 10, 4 March 2009, Pages 1485-1489