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ORIGINAL ARTICLE

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Assessment of Commercial Bivalent Inactivated Newcastle Disease Virus and Infectious Bronchitis Virus Vaccines against Prevalent Isolates in Egypt

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Abstract
Newcastle disease (NDV) and infectious bronchitis (IB) viruses are the most infectious and serious diseases on poultry flocks Egypt. Several types of bivalent inactivated NDV and IB vaccines are used to prevent and decrease incidence of both viruses but efficacy of these vaccines should be monitored periodically to understand the main causes of those breaks. In the current study nine bivalent inactivated NDV and IB vaccines were randomly selected from different batches during routine work at central laboratory for evaluation of veterinary biologics in 2023. Quality control of nine vaccines was applied according to Egyptian standard regulations for evaluation of veterinary Biologics. Completion of inactivation test of nine vaccines for both NDV and IB viruses revealed negative haemaagglutination test for NDV and normal appearance of embryo of IB virus when inoculation in SPF eggs. Immunization of one week old SPF chickens of nine selected vaccines showed neither clinical signs nor mortalities. Collection of serum samples from all tested vaccines on 3rd and 4th weeks post vaccination showed variable immune response against NDV using Haemagglutination inhibition test and ELISA test against IB virus. Challenge trial of tested vaccines showed variable percent of protection (50% to 100%). Virus shedding in SPF eggs on 3rd, 5th, 7th and 10th days post challenge revealed significant amount of virus in oropharengeal swabs. This study showed validation of some bivalent inactivated NDV and IB vaccines batches in vaccination programs and invalidation of other batches for use.

Keywords
Bivalent NDV and IBV Inactivated Vaccines, Genotype, Infectious Bronchitis Virus, Newcastle Disease Virus, Quality Control

1. Introduction
Infectious bronchitis virus (IBV) is an acute and highly contagious virus disease affecting all poultry flocks all over the world. Chickens and pheasants are natural host for IBV **(Dewit and Cook, 2019).** All ages of chickens are susceptible to IBV infection with induction of four clinical forms which are reproductive, renal, nervous and respiratory. The disease characterized by high morbidity, sneezing, nasal discharge drop in egg production with low quality and tracheal rales **(Milek et al., 2018)**. The virus is classified within family *Coronaviridae* (Gamma corona virus) measured 120nm in diameter with 20nm length crown like spike. It possess positive single stranded RNA of positive sense measured about 27.6Kb in length comprising 13 open reading frame **(Payne, 2017)**. The viral genomes composed of four structural proteins; nucleocapsid, envelope, membrane and spike besides PNA dependent RNA polymerase. S-Protein represents the most important one as it contributes in immunogenic and antigenic viral properties and furthered cleaved into S1 and S2 subunits, the subunit S1 glycoprotein is responsible for virus adsorption to receptor of cell and related to diversity of virus strains also induction of neutralizing antibodies whereas S2 subunit is associated with entry of virus to cell of host **(Lunge et al., 2016)**. Based on sequence analysis and phylogenetic tree, IBV is categorized into six genotypes including 32 viral linages **(Fraga et al., 2018)**. In Egypt, the first record of isolation and identification of IBV was in year 1954 and the virus was isolated in SPF eggs from chickens suffered from respiratory illness **(Ahmed, 1954)**. From that date till year 2000 investigations of IBV were applied relies on serological tests as serum neutralization test and agar gel precipitation test in addition to isolation of isolation of virus that showed circulation of IBV strains related to classic M41 and variant Dutch strains D274 and D1466 **(Taha et al., 1991)**. Partial sequence analysis of S1 gene showing identity of 99% and 97% to Dutch variants D274 and D3898 respectively and till that time the Dutch variants have not been reported **(Madbouly et al., 2002)**. Successful isolation of IBV from chickens suffered from renal and respiratory symptoms and characterization of S1 gene sequencing displayed that the virus was closely related to Israel with 97% similarity and this isolate known as Egyptian variant-1 **(Meir et al., 2004)**. Egyptian renal from of IBV strain isolated from broiler chickens with renal and respiratory

signs showing 97% similarity to H120 vaccinal strain using whole genome sequencing of S1 gene with correlation to GI-1 lineage but showed low protection using vaccinal strain H120 **(Selim et al., 2013)**. Two IB variant strains were identified as Eg/BSU-2/2011 and Ck/Eg/BSU-3/2011 and sequence analysis showed distinction from known any variant or vaccinal strains, these two variants designated as Egyptian variant-2 **(Abdel-Moneim et al., 2012).** Recently, Egyptian variant-1 and Egyptian variant-2 were identified as GI-23 lineage that represents the most predominant lineage in Egypt that has been circulating in all chicken flocks up till now **(Sabra et al., 2020)**. In 2012 new variant IBV strain VSVRI F3 was recovered from broiler flock with renal manifestations and the sequence analysis revealed 99% nucleotide identity to Q1 strain that was first recorded in china between 1996 and 1998 that later known as GI-16 lineage **(Abdel-Sabour et al., 2017).** In 2019 new pathogenic IBV was isolated from vaccinated layer flock and sequence analysis showed that 88-90% amino acid similarity with current vaccine group 793/B and designated as new lineage GI-13 lineage in Egypt **(Rohaim et al., 2019)**. Control strategy of IBV depends on using live and inactivated vaccines, inactivated vaccines are usually and common used for immunization of layer and breeder flocks to induce long-term humeral immunity during period of egg production besides transferring maternal immunity to offspring, the most common inactivated vaccines of IBV are M41 Ma5, H52 and H120 strains **(Cavanagh et al., 2003).** Newcastle disease is highly contagious, notifiable, and zoonotic with high commercial impact causing significant morbidity and mortality worldwide in poultry populations **(Dimitrov et al., 2017).** The etiological agent of disease is NDV virulent strains which are able to infect more than 250 species of birds. The virus is classified as avian *orthoavulavirus-1* in the family *paramyxoviridae*, *Avulavirinae* subfamily **(ICTV, 2019).** NDV genome is enveloped, single stranded RNA of negative sense, about 15kb encoding six genes; nucleocapsid, the phosphoprotein, the matrix protein, the fusion protein, the haemagglutinin-neuraminidase protein and the large polymerase and two non-structural proteins elicited by mRNA editing of P-gene **(Alexander et al., 2012).** Based on pathogenicity of disease all isolates of NDV are divided into five pathotypes: Velogenic (Viscerotropic or neurotropic) (highly pathogenic), Mesogenic (moderately pathogenic) and Lentogenic or respiratory (lowly pathogenic), and Asymptomatic enteric type **(Miller and Koch, 2013).** Genotypically, NDV is divided into classes I and II each including genotypes and sub genotypes; class1 strains comprises single genotype and 3 sub-genotypes while class II contains into 21 genotypes (I-XXI genotype XV consisting solely of recombinant genomes) **(Dimitrov et al., 2019a).** In Egypt the prevalent sub genotype of NDV is VIId (VII.1.1) which firstly recorded in 2011 and still causing devastating outbreaks **(Radwan et al., 2013).** Several outbreaks have been occurred in Egypt **(Osman et al., 2014; Awad et al., 2015; AbdEl-Aziz et al., 2016; Hagar et al., 2017; Mohamed et al., 2018; Mahmoud et al., 2019; Shakal et al., 2020).** Like IBV, control of NDV mainly depends on vaccination using live attenuated vaccines and inactivated oil adjuvant vaccines **(Alexander, 2017).** Mixed infection between NDV and IBV were declared among broiler flocks in Egypt with high mortality of more than 60% in some flocks **(Hussein et al., 2012).** Combined NDV and IBV vaccines are common used in poultry than using of separate vaccines not only to decrease cost, effort and number of vaccination performed in poultry industry but also protect against more than one disease at the same time **(Akeila et al., 2014).** Investigation of bivalent NDV and IBV imported and local vaccines in the protection against circulating wild type viruses revealed good immune response using HI and ELISA tests and high protection level against IBV and the virus could not be observed in internal organs after seven days post challenge using quantitative RT-PCR **(Walla et al., 2015).** The current paper primary aimed to evaluate the efficacy of commercial imported bivalent NDV and IBV in the protection against concurrent field strains in Egypt.

2. Materials and Methods

2.1. Ethical Approval

This study was approved by the Ethical Committee for Medical Research at the Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt in agreement with the local regulations and rules **(Approval number: 12020232/2022).**

2.2. Experimental Design

2.2.1. Vaccine Batches

Nine commercial imported bivalent NDV and IBV vaccine batches were randomly selected during daily routine work at central laboratory for Evaluation of Veterinary Biologics. The vaccines were introduced for the first time to the laboratory for quality control for registration before validation and approval. The vaccines batches were imported from china, introduced by 9 of different companies and their keys were kept with the authors. The vaccine numbers were coded from 1 to 9 **(Table, 1).**

Table 1. Designation of different bivalent IBV and NDV vaccines and the year of entry to laboratory for quality control.

Vaccine code	Designation of NDV and IBV vaccinal strains	Year of entry
1	NDV-CK-Danish/Pak-OP17-23+IBV-DE072 vaccine SP	2023
2	NDV-VRDC/Ventri/LaSota/Vaccine+ IBV- YX10 D90 vaccine	2023
3	NDV-RDC/Clone/LWS8/P3 + IBV-ArkDPI vaccine B-4	2023
4	NDV-VRDC/Clone/LM2/P3+ IBV-strain H120 vaccine	2023
5	NDV-VRDC/Clone/LWS8/P1+ IBV-Mass Vaccine 02- S1	2023
6	NDV-strain LaSota.71.IR+ IBV-strain 4/91 vaccine	2023
7	NDV-VRDC/Ventri/LaSota/WS+ IBV- GZ14/F80/vaccine	2023
8	NDV-VRDC/Clone/LM2/P1+ IBV-H120 - DS1	2023
9	NDV vaccine B1/BR+ IBV-Izovac-H120	2023

2.2.2. Specific Pathogen-Free Chicks and Eggs

A total of (360) three hundred and sixty one week old specific pathogen-free (SPF) chicks were obtained from the SPF egg production farm, Koum Osheim, El-Fayoum, Egypt. Fertile embryonated chicken SPF eggs of 9-11 day old were obtained from national project for production of SPF eggs (Koum Oshiem Fayoum, Egypt). The eggs were used for completion of inactivation test of tested vaccines and quantitation of virus shedding.

2.2.3. NDV and IBV Virus Strains

The local NDV genotype VIId circulating in Egypt (accession number KM288621) was used for completion of inactivation test and the same virus containing 10^6 egg infective dose 50/ml was used in challenge trial and designated as NDV-B7-RLQP-CH-EG-12 (accession number KM288609) **(Radwan et al., 2013).** The isolate was provided from reference laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt. Local IBV circulating in Egypt was used for completion of inactivation test (accession number KP729422) and the same virus containing 10^4 egg infective dose 50/ml used in

challenge trial designated as VSVRI_G9 **(Abdel-Sabour et al., 2017)**. The isolate was provided from poultry Department of Vaccines, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt.

2.3. Quality Control of Commercial Bivalent Inactivated NDV and IBV Vaccine Batches

All procedures of quality control of tested vaccines were applied according to **OIE (2018)** and **Egyptian Standard Regulation for Veterinary Biologics (2017).**

2.3.1. Completion of Inactivation Test

Random vaccine was selected from each supplied batches for completion of inactivation test. Briefly, 0.2ml of inactivated NDV vaccine was inoculated into five 9-11 day old SPF eggs at least for three passages via allantoic route parallel with five positive controls of NDV and IBV inoculated with the same dose and route. The eggs were incubated at 37°C with 40-60% humidity for seven days and candled daily. The completion of inactivation of the virus was detected by rapid slide HA test on the allantoic fluid harvested from inoculated eggs.

2.3.2. Immunization of SPF Chicks with Bivalent NDV and IBV Vaccines

Ten SPF chicks of one week old were vaccinated with tested bivalent NDV and IBV vaccine according to manufacturer's instructions.

2.3.3. Immune Response of Bivalent NDV and IBV Vaccines

Serum samples were collected at the third and fourth week post vaccination from five vaccinated chicks to determine antibody titer for NDV by HI test using reference antisera supplied from GD-Holland and antibody titer for IBV which can be calculated by reference to positive control that expressed as sample to positive ratio (S/P ratio) using ELISA kit.

2.3.4. Challenge Trial of Bivalent NDV and IBV Vaccines (Protection %)

Challenge trial was applied at the fourth week post vaccination for NDV using NDV genotype VIId containing 10^6 egg infective dose 50/ml and IBV virus containing 10^4 egg infective dose 50/ml. In challenge trial thirty SPF chicks of one week were divided into three groups:

1- Group A: includes ten SPF chicks of one week old that challenged with 100µl of NDV genotype VIId (accession number KM288609) via oculonasal route and were kept as positive control group.

2- Group B: includes ten SPF chicks of one week old that challenged with 100µl of IBV (accession number KP729422) via oculonasal route and were kept as positive control group.

3- Group C: includes ten SPF chicks of one week old that were kept as negative control.

Each group was kept in a separate isolator and monitored for 10 days to detect any clinical signs that may arise.

2.3.5. Virus Shedding of NDV and IBV Bivalent Vaccine

Oropharyengeal swabs were collected from vaccinated challenged chicks at the 3rd, 5th, 7th and 10th days post challenge besides positive control groups of NDV and IBV used in challenge trial for testing virus shedding. The virus quantitation was applied by inoculation of samples in 9-11 days SPF old chicks via allantoic route and virus titration was calculated according to **Reed and Muench (1938).**

All the above mentioned quality control procedures were carried out on all bivalent commercial inactivated NDV and IBV vaccines used in this paper.

3. Results

3.1. Completion of Inactivation Test

Inoculation of all tested bivalent commercial inactivated NDV and IBV vaccines in 9-11 day SPF eggs with 0.2ml via allantoic route revealed no deaths at the second day post inoculation. By Candling of eggs inoculated with NDV genotype VIId at the second day of inoculation revealed death of all positive control of SPF eggs embryos for all five eggs. Using rapid slide HA test to the allantoic fluid collected from the inoculated virus of positive control group showed positive agglutination to chicken erythrocytes. Positive control group inoculated with IBV demonstrated curling, dwarfing and deaths of all egg embryos at the end of incubation period. Negative control group revealed no deaths at the end of incubation period. Candling of inoculated SPF eggs of all tested bivalent NDV and IBV after the end of incubation period revealed no deaths and negative agglutination of allantoic fluid for NDV using rapid slide HA test and no abnormalities or curling of IBV embryos indicating proper inactivation of all tested vaccines.

3.2. Vaccines Immunization in SPF Chicks

Inoculation of all bivalent NDV and IBV vaccine batches in one week old SPF chicks revealed neither clinical signs nor mortalities at the end of incubation period.

3.3. Immune Response of Bivalent NDV and IBV

HI test for NDV of serum samples collected from vaccinated chickens revealed a good immune response based on the geometric mean (G.M) calculated from five chicken sera collected at interval times 3rd and 4rd, week post vaccination **(Table, 2).**

Table 2. Mean HI titers against ND virus in birds vaccinated with the tested ND vaccines on, 3rd, 4th weeks post vaccination (WPV).

Vaccine code	3 rd WPV	4th WPV	Vaccine code	3rd WPV	4th WPV
1	6 $\log_2$	6.6 $\log_2$	6	5 $\log_2$	5.8 $\log_2$
2	6.3 $\log_2$	7 $\log_2$	7	5.4 $\log_2$	6.2 $\log_2$
3	6.1 $\log_2$	6.8 $\log_2$	8	6.1 $\log_2$	6.9 $\log_2$
4	5.9 $\log_2$	5.5 $\log_2$	9	6 $\log_2$	6.7 $\log_2$
5	5.1 $\log_2$	6 $\log_2$			

ELISA test for IBV of serum samples collected from vaccinated chicken revealed variable immune response based on calculation of antibody titer by reference to positive control that expressed as sample to positive ratio (S/P ratio). Samples with S/P ratio 0.2 or greater are considered positive **(Table, 3).**

Based on serological response vaccine batches no 4, 5 and 6 are invalid due to inadequate immune response or immunogenicity induced by those vaccine batches.

3.4. Challenge Trial of Bivalent NDV and IBV Vaccines (Protection %)

Challenge trial of bivalent NDV and IB vaccines illustrated variations in protection level of vaccinated challenged chicks against NDV and IBV **(Tables, 4-5)**.

Positive control group inoculated with 100µl of NDV genotype VIId via oculonasal route developed NDV clinical signs from the beginning of the 3rd day post inoculation such as depression, inappetance, paralysis and twisted neck and all chicks died on the 7th day post inoculation while Positive control group inoculated with 100µl of IBV via oculonasal route developed IBV clinical signs such as respiratory rales, nasal discharge and all chicks died on 10th day post inoculation. No clinical signs or mortalities were appeared on negative control group along ten days observation period. Based on the results of protection percent vaccine codes numbers 5 and 6 are not valid for registration and approval as they induced insufficient protection level for NDV and IBV.

3.5. Virus Shedding

Titration of virus shedding from oropharyngeal swabs collected from vaccinated challenged chicks at the 3rd, 5th 7th and 10th days post challenge (DPC) in 9-11 day old SPF eggs inoculated via allantoic route against NDV and IBV revealed variable amount of virus load **(Tables, 6-7).**

Table 3. Antibody S/P ratio against IB antigen in tested bivalent inactivated vaccine on, 3rd, 4th weeks post vaccination (WPV).

Vaccine code	3rd WPV	Antibody titer	4th WPV	Antibody titer
1	0.28	1167.2	0.39	1625.7
2	0.29	1208.9	0.41	1709.1
3	0.27	1125.5	0.4	1667.4
4	0.2	833.7	0.31	1292.2
5	0.16	666.9	0.26	1083.8
6	0.1	416.8	0.11	458.5
7	0.19	792.0	0.21	875.4
8	0.24	1000.4	0.38	1584.1
9	0.21	875.4	0.31	1292.2

Table 4. Protection (%) of vaccinated challenged chicks on 4th week post vaccination (WPV) against NDV.

Vaccine code	Protection % (4th WPV)	Vaccine code	Protection % (4th WPV)
1	90%	6	70%
2	100%	7	90%
3	90%	8	90%
4	90%	9	90%
5	80%		

Table 5. Protection (%) of vaccinated challenged chicks on 4th week post vaccination (WPV) against IBV.

Vaccine code	Protection % (4th WPV)	Vaccine code	Protection % (4th WPV)
1	90%	6	50%
2	90%	7	90%
3	90%	8	90%
4	90%	9	90%
5	90%		

Table 6. Mean titer of viral shedding reduction from different vaccinated challenged chicks groups against NDV (oropharengeal swabs).

Vaccine code	Mean titer of reduction of viral shedding (log_{10}), DPC= day post challenge							
	3rd DPC		5th DPC		7th DPC		10th DPC	
	Vaccinated	control	Vaccinated	control	Vaccinated	control	Vaccinated	control
1	3	6	4.1	7.2	2	_	2	_
2	2.3	6	3.4	7.2	1.3	_	1.3	_
3	2.5	6	3.6	7.2	1.5	_	1.5	_
4	3	6	4.1	7.2	2	_	2	_
5	3.5	6	4.6	7.2	2.5	_	2.5	_
6	4.5	6	5.6	7.2	3.5	_	3.5	_
7	3.5	6	4.6	7.2	2.5	_	2.5	_
8	2.5	6	3.6	7.2	1.5	_	1.5	_
9	2.5	6	3.6	7.2	1.5	_	1.5	_

Table 7. Mean titer of viral shedding reduction from different vaccinated challenged chicks groups against IBV (oropharengeal swabs).

Vaccine code	Mean titer of reduction of viral shedding (log_{10}), DPC= day post challenge							
	3rd DPC		5th DPC		7th DPC		10th DPC	
	Vaccinated	control	Vaccinated	control	Vaccinated	control	Vaccinated	control
1	3.1	4.8	4.2	5	2.1	4	2.1	_
2	2.4	4.8	3.5	5	1.4	4	1.4	_
3	2.6	4.8	3.7	5	1.6	4	1.6	_
4	3.1	4.8	4.2	5	2.1	4	2.1	_
5	3.6	4.8	4.7	5	2.1	4	2.6	_
6	4.6	4.8	5.7	5	2.6	4	3.6	_
7	3.6	4.8	4.7	5	3.6	4	2.6	_
8	2.6	4.8	3.7	5	2.6	4	1.6	_
9	2.6	4.8	3.7	5	1.6	4	1.6	_

Positive control group of NDV and IBV tested for virus shedding of oropharyngeal swabs collected from vaccinated challenged chicks at the 3rd, 5th 7th and 10th days post challenge showed increase virus load from the 3rd to 5th days for NDV, death of all chicks at the 7th day post challenge while virus load was somewhat increase for IBV accompanied with death of all chicks on 10th day post challenge.

Based on the results of virus shedding inactivated bivalent NDV and IBV developed virus shedding 2Log$_{10}$ less than positive control groups are only approved and valid and so vaccine code number 6 was invalid for approval and registration.

4. Discussion

Infectious bronchitis and Newcastle disease are notifiable and most important viral disease of poultry sectors; both diseases affect all species and ages however, the disease degree differs according to age, infectious bronchitis induce sever manifestations in young chicks whereas Newcastle disease affects all ages of chickens **(Bande et al., 2016; Dimitrov et al., 2019b).** Both viral genome possess single RNA strand, hence evolution and genetic diversity are very high among circulating virus strains especially in case of infectious bronchitis virus where genetic recombination contributes to evolution **(Moreno et al., 2017).** Mixed infection between NDV and IBV is recurrent issue and this exaggerated without effective vaccination policies leading to difficulties in elimination of both diseases from poultry flocks consequently economic losses **(Hassan et al., 2016).** Vaccination and hygienic measures are the main effective approaches for controlling infectious bronchitis and Newcastle disease viruses, but these measures are hindered by continuous alterations and mutations among serotypes/genotypes that usually did not induce cross protection **(Tan et al., 2016).** Though live attenuated vaccines and inactivated are extensively used against NDV and IBV in Egypt but these vaccines did not establish immunity against IB and NDV in poultry flocks due to genetic and antigenic differences between predominant field virus strains and vaccinal strains **(Fawzy et al., 2020).** Generally, live vaccines are inexpensive, easy to inject and induce high titer but they have drawbacks such as virulence reversion and lower time of antibody titer. Killed vaccines little cost, produce good antibody titer that are kept for long period without influencing virus evolution **(Burgh and Siegel., 1978).** Separate vaccine inoculation whether live or inactivated may fail to produce proper protection level in vaccinated chicken; hence combination of two or than two vaccines became favorable for uses in poultry population in addition to reduce the cost of production, effort and time consuming for vaccination **(Cardoso et al., 2005; Akeila et al., 2014).** Continuous monitoring of currently used vaccines against concurrent field strains is extremely desired to ensure the efficacy of used vaccines, in this respect the current research was intended to assess the efficacy of imported commercial inactivated bivalent NDV and IBV.

In this research, random nine vaccines were selected from different batches during routine work at Central Laboratory for Evaluation of Veterinary Biologics in 2023; nine vaccines were coded from 1 to nine and were undergone for quality control for approval and validation **(Table, 1).**

The nine selected vaccines were tested for completion of inactivation test in SPF eggs and no residual viruses were detected after three blind passages indicating success of inactivation process during manufacture of vaccine procedures. Immunization of one week old SPF chicks with recommended vaccine doses showed no adverse reactions after ten days observation period. Serological response of the selected vaccines revealed variable antibody titers on third and fourth weeks post vaccination using HI test for NDV and ELISA for IBV **(Tables, 2-3).** In this study, HI test was used to detect antibody titers for vaccinated chicks to NDV vaccine as it is rapid and economic. Although HI test does not illustrate low titer of antibodies but it is pointer for immunogenicity of chicken flocks even with individual sera afterword vaccination **(Corbanie et al., 2008).** Also, ELISA test was used determine immune response to IBV vaccine, the test is sensitive and specific for deletion of antibodies for IBV **(Minta et al., 1990).** According to **OIE (2018)** and **Egyptian standard regulation for veterinary Biologics (2017)** vaccine codes 4, and 6 are not approved as their antibody titer less than 6 log$_2$ and S/P ratio less than 0.2 in vaccine code 6 Challenge trial of the selected nine vaccines revealed variable protection level ranged from 70% to 100% for NDV and 50% to 90% for IBV **(Tables, 4-5).** Likewise, challenge trial is used to expect chicken response to exposure to the concurrent field NDV and IBV **(Thonnton, 1988).** According to **OIE (2018)** and **Egyptian standard regulation for veterinary Biologics (2017)** vaccine codes 5 and 6 are not valid as protection percent less than 90%. Also, virus shedding is important measure to test efficacy of bivalent NDV and IBV vaccines **(Thonnton, 1988).** Based on **OIE (2018)** and **Egyptian standard regulation for veterinary Biologics (2017)** vaccine code number 6 was not approved and valid as shedding more than positive control group by 2 log$_{10}$ **(Tables, 6-7).**

In accordance with this study, using of combined inactivated NDV and IBV vaccines induce marked immune response at the 14th and 28th days and mean titers of 1601 and 1664 for IBV and 4.9 and 4.6 log$_2$ for NDV at the same days also, good protection against IBV after challenge with wild type IBV where the virus was not observed in kidney and trachea after one week post challenge using quantitative PCR **(Walla et al., 2015).** Combination of bivalent inactivated IBV lineages (M41; GI-lineage) and (VarII; GI-23 lineage) and NDV (genotype VII) (local strains) using oil adjuvant Montanide ISA-71 created potent, sterile and safe vaccine. This bivalent vaccine induces antibodies titers starting from first week post vaccination against NDV and IBV and reached maximum titer on sixth week post vaccination for IBV and forth week post vaccination for NDV. Also, the developed vaccine revealed complete protection against both virulent viruses **(Shawky et al., 2020).** Secondary boosters of bivalent inactivated NDV and IBV elicit immune response up to ten weeks in comparison with monovalent forms. This response was exaggerated when followed by live primary vaccination with live vaccine combined of both NDIB primary vaccines **(Gough et al., 1977).** On the other hand, bivalent IBV and NDV vaccines might not create immunity against IBV and NDV due to interference of infectious bronchitis vaccine with NDV vaccine response if IBV titer is increased during preparation process **(Cook, 2008).** Also, **Cavanagh and Naqi (2003)** showed that if titer of IBV is more than NDV interference may occur. This interference not only fails to induce sufficient immune response but also no appropriate protection will be achieved against challenge with wild type virus accompanied with high levels of stress **(Winterfield, 1984).** On the contrary **Masoudi and Ebrahimi (2017)** observed that no of IBV vaccine on NDV immune response and strong immune response was elicited in vaccinated chickens. Likewise, No interference was observed of IBV vaccine on NDV immune response when vaccine formulated in laboratory not just vaccine association before vaccination that help in interference of virus **(Smith, 2002).**

In addition, using bivalent IBV and NDV vaccines reduce immune pressure caused by recurrent injections, less mortality in populations and eradicating harmful agents. Moreover, diminishing the presence of vaccinators and post vaccinal reactions accompanied with complications **(Masoudi and Ebrahimi, 2017).**

Overall, it is favorable to control NDV and IBV epidemics in a one vaccine dose subsequently, that will save time, labor and diminish stress immunity during vaccination of susceptible chicken.

5. Conclusion

Continuous evaluation of vaccination programs against IBV and NDV are required to ensure their effectiveness against concurrent

field strains and importance of surveillance of NDV and IBV to characterize new evolving strains.

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7. Authors Contributions

El-Safty MM and Zaghloul MA designed the practical experiment, Attya HM, ElSayed MF and El-Dabae WH applied the practical work. All authors revised and approved the final manuscript.

8. Conflict of Interest

All authors declared no conflict of interest

9. References

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