

## Comparison between Local and Imported Vaccine against Newcastle Disease, Infectious Bronchitis and Egg Drop Syndrome

Akeila MA<sup>1</sup>, Khalil S A<sup>1</sup> and Sedeik M<sup>2</sup>

<sup>1</sup> Microbiology Dept. Fac. Of Vet. Med. Alex. University

<sup>2</sup> Poultry and Fish Diseases Dept. Fac. Of Vet. Med. Alex. Univ

\*Corresponding author's e-mail: [Seddeeklab@yahoo.com](mailto:Seddeeklab@yahoo.com)

**ABSTRACT:** The aim of this study is to, prepare a potent inactivated pentavalent combined vaccine from ND, IB and EDS, evaluation the immune response of chickens to the locally prepared vaccine and study the comparative efficacy of the locally prepared pentavalent vaccine with the imported one. In this study, 270 SPF chickens were used to evaluate the efficacy of different imported and locally prepared inactivated pentavalent vaccines. The birds were divided into 3 experimental groups 1, and 2 (100 birds /each), each group was divided into 4 subgroups while Control group (3) had 70 birds and sub-divided into 4 subgroups. The viruses used in this study includes newcastle disease virus strain, the vaccinal strain was the lentogenic living LaSota strain and received from Department of Animal Science and Agricultural Biochemistry, University of Delaware, New York, USA. The titer was 10<sup>10</sup> EID<sub>50</sub> / ml. While, the challenge strain was virulent velogenic viscerotropic locally isolated field strain of Newcastle disease virus. It was isolated, identified and typed. Its titer was 10<sup>9</sup> EID<sub>50</sub> in embryonated chicken eggs. The infectious Bronchitis virus, it was classical Massachusetts 41 (M-41) typed and received as allantoic fluids from Department of Animal Science and Agricultural Biochemistry, University of Delaware, New York, USA. Its titer was 10<sup>8.5</sup>EID<sub>50</sub> / ml. nut, the egg drop syndrome virus was strain EDS 76 typed and received as allantoic fluids from Department of Animal Science and Agricultural Biochemistry, University of Delaware, New York, USA. Its titer was 10<sup>7</sup> EID<sub>50</sub> / ml. So the conclusion was that the local pentavalent vaccine (ND, IBV, EDS) gave acceptable antibody titers and good protection levels in comparison with the imported pentavalent vaccine.

**Key Words:** Comparison, Local, Imported Vaccine, Newcastle Disease, Infectious Bronchitis, Egg Drop Syndrome

ORIGINAL ARTICLE

Received 9 Jan. 2014  
Accepted 10 Mar. 2014

### INTRODUCTION

Combined vaccines have the advantage of protection against more than one disease at the same time, beside, reducing vaccination expenses, number of vaccination performed and saving time. The most important problems facing the poultry industry in Egypt are the infection with avian Newcastle disease, Infectious bronchitis and egg drop syndrome. They cause economic losses, particularly in those parts of the world where the poultry industries are intensive and where open sided housing is common.

Poultry producing areas of many parts of the world have been threatened by a widespread increase in the number of severe epizootics of velogenic Newcastle disease [1]. The poultry industry contains to be plagued by these diseases and losses are mainly due to elevated mortality and reduced weight gains, together with reduced egg production in layers, and nervous manifestations and air sacculitis condemnation in broilers [2].

Infectious bronchitis virus (IBV) is one of the primary causes of respiratory disease in domestic fowl. Infection with IBV reduces the performance of broilers and in laying birds drops in egg production and egg quality can occur [3, 4].

Infectious bronchitis (IB) is an acute, highly contagious respiratory, renal, and urogenital disease of chickens caused by the coronavirus. Infectious bronchitis virus (IBV).it is still a major health problem in the chicken industry worldwide [5].

Egg Drop Syndrome (EDS) is the major cause of loss of egg production throughout the world. It is posing a serious threat to layer industry worldwide. The EDS is caused by hemagglutinating duck adenovirus-I which is a DNA virus. Recently, EDSV has been placed in a new genus at adeno virus of family adenoviridae [6, 7].

The aim of this study is to, prepare a potent inactivated pentavalent combined vaccine from ND, IB and EDS, evaluation the immune response of chickens to the locally prepared vaccine and study the comparative efficacy of the locally prepared pentavalent vaccine with the imported one.

## MATERIAL AND METHODS

**A.Experimental Design:** In this study, 270 SPF chickens were used to evaluate the efficacy of different imported and locally prepared inactivated pentavalent vaccines. The birds were divided into 3 experimental groups 1, and 2 (100 birds /each), each group was divided into 4 subgroups while Control group (3) had 70 birds and sub-divided into 4 subgroups as shown in the following figure.

### **B.Strain used:**

- Bacterial strains
- Salmonella enteritidis:

It was taken and prepared locally in the Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, and Cairo. The titer was 10<sup>8</sup> CFU / ml. These strains were used in vaccine and antigen preparation as well as in challenging vaccinated chickens.

- Avibacterium paragallinarum

Serotypes A, B and C [1] were prepared locally in the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The titer of each was 3X10<sup>8</sup> CFU / ml. These strains were used in vaccine and antigen preparation as well as in challenging vaccinated chickens.

### **c. Laboratory chickens:**

Specific pathogen free (SPF) chickens were obtained from Khom Oshem farm, El Fayoum, Egypt as one day old. They were reared and housed in positive pressure stainless steel isolation cabinets at CLEVB with continuous light exposure till used.

### **D.Culture media:**

The following media were used For Salmonella Enteritidis:

- Salmonella Shigella agar medium (Oxoid ltd. Basing stake, Hampshire, England).
- Tryptose soya agar medium (Oxoid Ltd. Basing stake, Hampshire, England).

The media used for Avibacterium Paragallinarum:

- Tryptose phosphate broth (Code No. 0060 - 01 - Difco Laboratories, Detroit, Michigan, USA). It was used for rehydration of lyophilized strain.

Brain heart infusion broth (Code No. 152 - 00680 M - Difco LTD, Paisley, Scotland)

### **E.For testing vaccine sterility:**

The different vaccines were inoculated on the following media for performing the sterility tests for bacteria and fungi according to the British pharmacopeia [8].

**a)** Thioglycolate broth for anaerobic bacteria which was inoculated and incubated at 37C for 72hours.

**b)** Nutrient agar plates for facultative anaerobic and aerobic bacteria which was inoculated and incubated at 37C for 72 hours.

**c)** Sabouraud's maltose broth and Sabouraud's maltose agar plates to test fungal sterility which was inoculated and incubated at 25C for 14 days.

**1.** Preparation of bulk culture from *S. enteritidis* according to Charles et al. [8].

**2.** Inactivation of *S. enteritidis* [8].

**3.** Safety (completion of inactivation for *S. enteritidis*)

It was carried out by culturing at nutrient broth to detect the presence or absence of growth and on sure complete inactivation.

### **f. Avibacterium paragallinarum:**

**1.** Preparation of bulk culture from Avibacterium paragallinarum.

**2.** Inactivation of avibacterium paragallinarum cultures.

**3.** Safety (completion of inactivation for A. paragallinarum).

It was carried out by culturing at tryptose phosphate broth to detect the presence or absence of growth and on sure complete inactivation.

### **G.Evaluation of the produced vaccines:**

It was done according OIE.

#### **-Identity:**

The identity of every component incorporated in the vaccine under test is carried out through testing of sera collected from vaccinated chickens (in conjunction with potency test).

#### **-Completion of inactivation:**

Ten SPF 9-11 day old ECE were inoculated each with the recommended dose of inactivated vaccine. The inoculated eggs were candled twice daily for six

Days the embryos that died after within first 25 hours were discarded. The embryos that died after the first 24 hours as well as the survivors, 6 days post inoculation were tested for the presence of haemagglutination activity using the rapid HA test. The harvested fluids were blindly passaged for 2 other passages before the vaccine being emulsified.

**-Sterility:** It was carried out according to Allan et al. The prepared pentavalent inactivated vaccine was inoculated into different types of media for sterility testing:

a. Thioglycolate broth was inoculated and incubated at 37°C for 72 hours.

b. Nutrient agar plates were inoculated and incubated at 37°C for 72 hours.

c. Sabouraud's maltose broth and Sabouraud's maltose agar plates were inoculated and incubated at 25°C for 14 days. [9].

**-Extraneous agent:**

The test was run in conjunction with safety test. After 3 weeks, each inoculated bird with double dose was inoculated S/C with another one field dose from the tested vaccines. Serum samples were collected two weeks later and tested for antibodies to extraneous viral agents were performed.

**Safety:**

It was done according to [9].

Three groups of ten SPF chickens, one day old, were injected S/C with two doses from each tested vaccine. The vaccinated birds were observed 21 days post vaccination for the general appearance (behavior, appetite, and development status as weakness, dropping or any unexpected adverse events), body weight, performances and macroscopic examination of injection site

**Potency:**

It was done to demonstrate the antigenic capacity for each tested vaccine. SPF chickens, four weeks old, were vaccinated S/C with field dose. Blood samples were drawn weekly for 6 weeks (30 sample per week) and the serum samples were separated, inactivated at 56°C /30min and kept at -20°C till used. The serological analysis was done to antibody level against each component of the tested vaccines. Also, at 21 days post vaccination, 150 birds were challenged by local and virulent isolates corresponding to the vaccine component. The morbidity and mortality rates were recorded for each group till the end of the observation period to measure the protection %.

**B. ELISA for IB and S. enteritidis using kits (biocheck kit) Haider et al. [10]**

**Reagent preparation: The microtiter plates coated with antigen were**

Calculation of S/P ratio:

Calculation of Antibody Titer:

SP ratio

$\text{Log}_{10} \text{Titer} = 1.13 \text{Log} (\text{SP}) + 3.156$

$\text{Antilog} = \text{Antibody titer.}$

## RESULTS AND DISCUSSION

Results of Haemagglutination Inhibition titers for ND in sera of chicken vaccinated with local and imported inactivated polyvalent Penta vaccines are described in Table (1). The local and imported inactivated Penta vaccinated chickens showed an increased HI level till 6th WPV as compared with the control. The present results are consistent with those of [11].

Inactivated Newcastle disease vaccines are used extensively in most parts of the world. The major advantage of inactivated Newcastle disease vaccines is that they do not produce the undesirable side effects sometimes associated with live virus vaccines [12]. Also, they may be better suited in combined viral and bacterial vaccines in order to circumvent some of the flock's problems.

The Haemagglutination Inhibition test is the most convenient, rapid and economical serological method for evaluating the immunity of chicken to Newcastle disease virus. Although, the HI test generally does not detect low levels of circulating antibodies, it has proved to be an indicator of the immune status of a flock when individual sera are tested after vaccination [13].

Challenge under strictly controlled conditions with virulent NDV strain may also be used to predict flock response to exposure. This method combined with serologic findings can add considerable significance to the HI values obtained with sera from the same chicken. These findings substantiate the results.

From data recorded in Table (2), it is observed that vaccination of chicken with local and imported Penta inactivated vaccine then challenged with virulent strain of Newcastle disease virus evoked a high degree of protection reaching 93.4% and 100 %, respectively. It could be deduced that imported vaccines gave a highly acceptable protection level in comparison with local vaccine. These results agreed with previous findings [14].

Also, the present work describes the serological response to vaccination against IB virus experimentally either with local or imported Penta inactivated polyvalent vaccines containing Infectious Bronchitis component. The ELISA test was used to assess the antibody response to the Infectious Bronchitis component of the vaccines. As shown in Table (3), the chickens were all found to have IB antibody titers reaching 156.2 before their vaccination. This finding confirms the suggestion that IB virus was active in all flocks of SPF chickens at and before the time of vaccination [15]. After the first week post vaccination, there was a significant rise in the ELISA antibody titers in the two groups of chicken receiving local and imported Penta inactivated IB vaccines than those of the control group. By 6th WPV, the ELISA antibody titers reached at higher levels among all vaccinated groups. There was a slight increase among group of chicken receiving imported Penta IB vaccine (6366.8) than by those receiving local vaccine (6268.6). Thus, the tested IB vaccines induced high and sustained immunity to IB disease and protect the chicken against infection with IB virus. These observations supported [16].

From the results shown in Table (4), it appears that the mean HI titers of EDS antibody level raised from 0 at prevaccination till reaching the maximum level (8 log<sub>2</sub>) at the 5th week post vaccination then persisted similar up to the 6th week post vaccination among groups of chicken receiving local Penta inactivated vaccine. If compared with those receiving imported Penta vaccine where they showed higher levels of HI titers from the beginning of fifth WPV and also persist constant till the 6th WPV. The high level of immunity against EDS produced by local and imported inactivated Penta vaccines confirmed earlier work [8] and showed that inactivated multivalent combined vaccines could be administered in local or imported form without loss of potency inducing a satisfactory immune response to EDS virus when evaluated under laboratory condition.

So the conclusion was that the local pentavalent vaccine (ND, IBV., EDS) gave acceptable antibody titers and good protection levels in comparison with the imported pentavalent vaccine.

**Table 1.** Mean HI antibody titer against ND component of the local and imported inactivated polyvalent Penta vaccines in the sera of vaccinated birds

Groups	Type of vaccines	No. of serum samples	HI antibody titers (Weeks post vaccination )						
			Pre.	1	2	3	4	5	6
1	<b>Local</b>	10	0	5	5.2	6.5	7.3	8.0	8.2
2	<b>imported</b>	10	0	4.9	5.4	6.3	7.1	7.8	8
3	<b>Control</b>	10	0	0	0	0	0	0	0

**Table 2.** Results of the efficacy of the local and imported inactivated polyvalent Penta vaccines in vaccinated birds against the challenge with local strain of VVND virus at 3 weeks post vaccination

Groups	Type of vaccines	No. of birds	Daily examination for deaths (days post challenge)										Total deaths	Protection %*
			1	2	3	4	5	6	7	8	9	10		
			1	<b>Local</b>	15					1				
2	<b>imported</b>	15										0.15	100	
3	<b>control</b>	10			6	4						10.10	0	

\*Minimum protection % is 90% according Egyptian regulation

**Table 3.** Mean ELISA antibody titer against IB component of the local and imported inactivated polyvalent Penta vaccines in the sera of vaccinated birds

Groups	Type of vaccines	No. of serum samples	ELISA antibody titers (Weeks post vaccination )						
			Pre.	1	2	3	4	5	6
1	<b>Local</b>	10	156.2	1281.8	3156.6	4343.3	5178	5581.6	6268.6
2	<b>imported</b>	10	156.2	1389.2	3301	4388.5	5277.8	5693	6366.8
3	<b>control</b>	10	156.2	164	175.6	187	201	214	360

Positive ELISA titre range: 834 or greater according to ELISA kit.

**Table 4.** Mean HI antibody titer against EDS component of the local and imported inactivated polyvalent Penta vaccines in the sera of vaccinated birds

groups	Type of vaccines	No. of serum samples	HI antibody titres (Weeks post vaccination )						
			Pre.	1	2	3	4	5	6
1	<b>Local</b>	10	0	4.7	5.5	7.1	7.8	8	8
2	<b>Imported</b>	10	0	5	6	7.5	8	8.1	8.1
3	<b>Control</b>	10	0	0	0	0	0	0	0

## REFERENCES

1. Beard, C.W. & Brugh, M.Jr. 1975. immunity to Newcastle disease. Am. J.Vet. Res, 36: 509-512.
2. Halvorson, D.A., Shaw, D., Sivanandan, V., Barbour, E.K., Maheshkumar, S., Newman, J.A. & Newman, L. 1991. serological response in borilor chicks to different commercial newcastle disease and infectious bronchitis vaccines. Avian Dis, 35:978-981.
3. Cavanagh, D.S., Calnek, B.M., Bames, H.J., Macdougald, L.R. & Saif, Y.M. 1997. infectious bronchitis in Nagi . Disease of poultry, 511-526.
4. Allen, W.H; Lancaster, J.E. & Toth, B. 1973. The production and use of Newcastle disease vaccines. F.A.O, Rome, ITALY, 1115, 53
5. Rosie, M., Ora, M., Ygal, F. & Lubov, S. 2010. Development of a real-time TaqMan® RT-PCR assay for the detection of infectious bronchitis virus in chickens, and comparison of RT-PCR and virus isolation. Journal of Virological Methods, 163:190-194.

6. Yamaguchi, T., Blackall, P.J., Takigami, S., Iraitani, Y. & hayashi, Y. 1991. Immunogenicity of haemophilus paragallinerium serovar B strains. *Avian, Dis*, 35 (4): 965-968.
7. Badar, S. T., Siddique, M., Ali, R. & Rasool, M. H. 2006. serological status of egg drop syndrome in breeders and commercial layers in mansehra district.pakistan vet. J, 26(1): 33-35.
8. Charles, S.D., Hussein, I., Nagraja, K.V. & Sivanadan, V. 1994. Adjuvanted subunit vaccines for the control of Salmonella enteritidis infection in turkeys. *Am. J. Vet. Res.*, 55 (5): 636-642.
9. Sayed, R.H. 2010.Preparation and evaluation of combined inactivated vaccine against S. enteritidis, S. typhimurium, C. perfringens type A and C toxins in chickens. Master. Sci. Thesis, Fac. Vet. Med., Cairo Univ. Shouman, M.T. and Moustafa, F. (1972): Salmonellosis in chickens. *Vet. Med. J.*, 20: 183 -192.
10. Haider, M.G., Rahman, M.M., Hossain, M.M., Rashid, M., Sufian, M.A., Islam. M.M. & Haque, A.H. 2007. Production of formalin killed fowel typhoid vaccine using local isolates Salmonella gallinarium in Bangladesh. *Bangl. J. Vet. Med*, (5): 33–38.
11. Mohamed, A.M. 1996. Trials for preparation and evaluation of a combined poultry vaccine composed of Newcastle disease virus and hemophilic paragallinarium. PhD. Sci. Thesis, Fac. Vet. Med., Cairo Univ OIE (2008): Evaluation of the produced inactivated vaccines.
12. Winterfield, R.W., Thacker, H.L. & badylack, S.F. 1984.Effects of subtype virations of the Holland strain of infectious bronchitis virus when applied as vaccine. *Poultry Sci*, 63 (2): 246-250.
- 13.Yamamoto, R. 1984.In: disease of poultry, 8th Ed. M.S. Hofstad, H.J. Barnes, B.W. Calnek, W.M. Reid and H.W. Jr. Yoder, Iowa, State Univ. Press, Iowa USA, 178-186.
14. Page, L.A., Rosenwald, A.S. & Price, F. C. 1963. Haemophilus infectious in chickens. IV. Results of laboratory and field trials of formalized bacterins for the preventions of disease caused by Haemophilus gallinarium. *Avian. Dis.*, 7: 239-256.
15. Marius, V., Gillet, J.P., Hospitalier, R., Guittet, M. & Bennnejan, G.1983. Vaccination against infectious bronchitis in chicks, whether or not bearing maternal antibodies. *Comp. Immunol. Microbiol. Infect. Dis.* 6:115-134.
16. Boots, M.H. 1991.the cellular immune response to infectious bronchitis virus. Amodel for development of a peptide based vaccine. Thesis, Rijksuniversiteii to Utrecht, Netherlands, 132.