Comparative study on immunogenicity of different inactivated Newcastle disease vaccines used in Egypt and role of prebiotics in improve immune response of commercial broiler chickens.

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Abstract: This study was conducted to evaluate immunogenicity and protective efficacy of different inactivated Newcastle disease (ND) vaccines given to 10 days old chicks with or without prebiotics. Both weekly sera for haemagglutination inhibition (HI) test and tissue samples for histopathology were applied while challenge test with virulent field virus were carried out at the end of the 3rd week post vaccination. In this study; 360 1-day old commercial broilers were grouped into 12 equal groups (30 chicks each). Chicken groups were vaccinated by Hitchener B1 vaccine at 5 days of age for priming followed by subcutaneous (S.C.) injection with inactivated ND vaccines as follow: groups (1& 6); (2& 7);(3& 8); (4& 9) and (5& 10) were received monovalent inactivated Locally produced ND vaccine ND-SVRI; Volvac® ND-AI; Volvac® ND Conc. KV; Laprovet® as well as Imopest® vaccine; respectively. While groups 11 and 12 kept as positive control prebiotics and negative control groups; respectively. Groups 6, 7, 8, 9 and 10 were treated with commercial prebiotics from day one old. All vaccinated groups showed no clinical signs or mortalities, after challenge with field virulent virus control negative and those received prebiotics showed clinical signs and mortalities of 90% and 80% respectively. HI test result in groups treated with vaccine and prebiotics are high. By the end of 3rd week post vaccination, the highest HI-titer were $\log_2$ 9 in groups receive Volvac® ND and Imopest® followed by groups receive bivalent Volvac® ND-AI and Laprovet® which was 8.7, finally it was 8.2 for group received ND SVRI. Groups received prebiotics alone and control negative showed titres of 2 and 3 by the end of 3rd week post vaccination; respectively. Vaccinated groups received prebiotics have positive effect on antibody titer for all vaccinated groups. Antibody titer was decreased sharply in all groups at 1 weeks post challenge. Groups receive vaccine without prebiotics was nearly 1 log lower than those received vaccine with prebiotics. Bursal sections of all vaccinated groups showed varied degree of histopathological changes from one vaccine to another as it showed slight lymphoid follicle depletion in groups received monovalent ND-SVRI and monovalent ND Laprovet®, while groups received bivalent Volvac® ND-AI and Volvac® ND Conc. KV showed moderate lymphoid depletion together with hyperactivity of the lymphoid follicle, while group received ND Imopest® showed neutrophilia together with hyperactivity of lymphoid follicle that shows even activated germinal center with presence of lymphoblast and mitotic. Groups received inactivated vaccines together with prebiotics showed bursa with light lymphoid follicle depletion but normal spleen and thymus. After challenge lesion in bursa of all groups showed changes varied from sever bursal depletion to sever bursal depletion with connective tissue formation, spleen showed depletion with congestion of red pulp, there was no pathological changes in thymus. It could be concluded that priming vaccination of chicks with live ND vaccine followed by inactivated Newcastle vaccine produce good immune response protect birds from challenge, moreover the use of prebiotics found to be of value in improving HI titers against Newcastle. The use of prebiotics to improve immune response to used Newcastle vaccines is important.

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Key words: inactivated ND vaccines, bivalent AI and ND vaccine, broiler chickens, HI test, challenge test, prebiotic, histopathology.

1. Introduction

Newcastle disease (ND) is one of the most important poultry disease causing severe economic losses in poultry industry, etiological agent is Newcastle disease virus member of Genus Avulavirus, family Paramyxoviridae ***(Mayo, 2002)***. On basis of infection severity in chicken Newcastle disease virus classified into three pathotypes (lentogenic, mesogenic and velogenic), moreover virus infection can manifest Newcastle pathotype result in high mortality in broilers chickens and sever drop in egg production (***Leslie, 2000 and Musa et al., 2010)***, this emerged the need for strict biosecurity measures together with vaccination program against this lethal disease. Most of countries

where poultry is raised commercially either broiler or layer or breeders and where is the disease is endemic rely on different vaccination programs on order to control the disease ***(Alexander and Senne, 2008)***. In spite of use of many vaccination programs include both commercial live and inactivated oil adjuvant vaccines ***(Al-Zubeedy, 2009)***, there is continued outbreak of velogenic Newcastle disease in reared poultry farms which give raise for need of more effective vaccination program in protecting birds against challenge ***(Van Boven et al., 2008)***. Many serological tests are useful tool for assaying the antibody responses of commercial broiler chickens vaccinated against ND such as HI test and ELISA test ***(Cevelic-Cabrilo et al., 1993)***. Many researchers pointed out that birds vaccinated with inactivated Newcastle disease vaccine showed minimal pathological changes, while those non immune birds challenged with virulent field virus showing sever histopathological changes ***(Mohammadamin and Qubih, 2011)***. From the above mentioned data this study was designed in order to examine the immune response together with protection against challenge and histopathological changes caused by different inactivated Newcastle vaccines alone or combined with prebiotics against challenge with field Newcastle virus.

2. Materials and Methods

Experimental birds:

Three hundred and sixty (360), one day old, broiler chicks were fed commercial ration and reared under strict hygienic measures.

Inactivated ND Vaccines:

1. Monovalant ND inactivated vaccine manufactured by Serum and Vaccine Research Institute (SVRI).
2. Bivalent ND-AI Volvac® (Borhinger Ingelhium) inactivated vaccine: batch no. 1209009A.
3. Monovalant Volvac® ND Conc. KV (Borhinger Ingelhium) inactivated vaccine: batch No. 1207068A.
4. Monovalant Laprovet® ND inactivated vaccine: batch no. 0603BG1A.
5. Monovalant Imopest® ND inactivated vaccine (Merial): batch no. L393730.

Live ND Vaccines

Hitchener B1 live attinuated vaccine "Hypervet": Batch number 5045063.

Prebiotics "Hydroenzime"®

Produced by AGRANCO – USA – Miami, Batch no. WIN-100/11 – Exp. Date 08/07/2014. It was used according to manufacturer instructions (1 gm/ 3 liters drinking water).

Virulent ND virus:

Used for challenge broiler chickens throughout the experiments was a locally field isolate, velogenic visrotropic Newcastle disease virus (VVNDV). It was isolated and identified by ***Sheble and Reda (1976)***. Kindly supplied by Egyptian Serum and Vaccine Research Institute with infective titer of 10^8 ID_{50}/ml.

Estimation of virus infectivity:

Infectivity of used challenge virus was done according to ***Anon (1971)*** and the embryo infected dose 50 (EID_{50}) was calculated according to ***Reed and Muench (1938)***.

Haemagglutinating antigen:

It was prepared according to methods of ***Allan et al. (1973).***

Serum samples for HI test:

Clotted blood samples were individually collected from wing vein for serum according to ***Anon (1971)***.

Chicken red blood cells:

Red blood cells (RBCs) from susceptible adult birds were collected on 4% sodium citrate as anticoagulant. The RBCs were washed three times with phosphate buffered saline (PBS) at PH 7.0 – 7.2.

Tissue samples for histopathology:

Tissue specimens from bursa, spleen and thymus of experimentally infected, vaccinated and control chicks were fixed in 10% neutral formalin solution for histopathological examination.

Haemagglutination inhibition (HI) test:

The test was carried out according to the standard procedure described by ***Majiyagbe and Hitchner (1977)*** the end point were estimated according to scheme described by ***Kaleta and Siegmann (1971).***

Histopathological studies:

Tissue specimens were fixed in 10% neutral formalin solution and the specimens were routinely processed in paraffin embedding method, sectioned and stained with Haematoxylin and Eosin for light microscope examination according to method described by ***Bancroft and Gamble (2008).***

Experimental design:

360 1-day old commercial broilers were grouped into 12 equal groups (30 chicks each). Chicken groups were vaccinated by Hitchener B1 vaccine at 5 days of age for priming followed by subcutaneous (S.C.) injection with inactivated ND vaccines at 10 days of age as follow: groups (1& 6); (2& 7);(3& 8); (4& 9) and (5& 10) were received monovalent inactivated Locally produced ND vaccine ND-SVRI; Volvac® ND-AI; Volvac® ND Conc. KV; Laprovet® as well as Imopest® vaccine; respectively. While groups 11 and 12 kept as positive control prebiotics and negative control groups; respectively. Groups 6, 7, 8, 9 and 10 were treated with commercial prebiotics from day one old. Both sera for haemagglutination inhibition (HI) test and tissue samples for histopathology were weekly collected while challenge test with virulent field virus were carried out at the end of the 3rd week post vaccination.

3. Results and Discussion

All groups received vaccine alone or with prebiotics shows no mortalities and clinical signs including those control negative and control received prebiotics only, after challenge with field virulent virus control negative and those received prebiotics showed clinical signs and mortalities of 90% and 80% respectively, this result was matched with result obtained by ***Chansiripornchai and Sasipreeyajan (2006)*** who found that all chickens group non vaccinated against ND virus and challenged with field ND virus showed 100% mortality, indicating that there was no disease resistance of this unvaccinated control group.

Table (1) shows result of HI test in groups treated with vaccine and prebiotics which revealed that all used vaccines are immunogenic, stimulate antibody production start from first week post vaccination for all groups, antibody titer was protective by the end of 3rd week post vaccination, the highest titer were 9 in groups received Volvac® ND and Imopest® followed by groups received bivalent ND-AI Volvac® and ND Laprovet® which was 8.7, finally it was 8.2 for group received ND - SVRI, both groups received prebiotics alone and control negative were not protective and start zero at first week and become 2, 3 by the end of 3rd week post vaccination respectively.

Vaccinated groups received prebiotics showed that prebiotics have positive effect on antibody titer for all vaccinated groups through all experiment when compared with those groups received inactivated vaccine alone. one weeks post challenge antibody titer decreased sharply in all groups as in groups receive vaccine without prebiotics was (4.7) in group received Imopest® followed by Volvac® ND conc KV (4.5) then groups received ND Laprovet (4.1) then groups received Bivalent ND-AI Volvac® (4) and finally (3) in groups received ND - SVRI. In case in groups received inactivated vaccine together with prebiotics HI titer were 5, 5, 4.7, 4.5 and 4.4 in groups received Imopest®, Volvac® ND Conc, Bivalent ND-AI Volvac®, ND - SVRI, ND Laprovet® respectively.

Table (1): Result of HI test in groups treated with vaccine and prebiotics.

Week	Control negative gp. 12	Prebiotics alone gp. 11	ND – SVRI		Bivalent ND-AI Volvac®		Volvac® ND conc KV		ND Laprovet®		ND Imopest®	
			alone gp. 1	with prebiotics gp. 6	alone gp. 2	with prebiotics gp. 7	alone gp. 3	with prebiotics gp. 8	alone gp. 4	with prebiotics gp. 9	alone gp. 5	with prebiotics gp. 10
1	0	0	0	2	2	3	2	3	0	0.3	2	3
2	1	2.5	6	7.5	6	7.5	6.5	8	7	8	7	8.5
3	2	3	8.2	10	8.7	10	9	10	8.7	9	9	9.5
4	2.5	3.5	3	4.5	4	4.7	4.5	5	4.1	4.4	4.7	5

Results of protection caused by used vaccine were parallel with that of ***Liljebjelke et al (2008)*** who stated that HA inhibition titers > 2^5 log2 would provide 100% protection from morbidity and mortality and require a minimum protective dose of 1000 HAU per bird this explained the protection caused by used vaccines against challenge. Moreover, ***Chansiripornchai and Sasipreeyajan (2006)*** found that combination of live vaccine and inactivated oil adjuvant Newcastle vaccine protect against challenge, this may due to production of local mucosal immunity by live vaccine prevent virulent field virus natural infection together with slow antigen release from inactivated vaccines behave as a booster dose ***Folitse et al (1998)***. Variation in antibody titer from inactivated vaccine to another maybe due to adjuvant used in each one, as it was found that some adjuvant used in activated ND vaccine may increase antibody titers in blood ***Marina et al (2014).*** In case of those groups received inactivated vaccine together with prebiotics there was improvement in antibody titer which was parallel with result obtained by ***Jennifer et al (2011)*** who stated that oral treatment of chickens with Lactobacilli has positive effect on both systemic antibody and cell mediated immune response, same result was obtained by ***Landy and Kavyani (2013)*** who found that use of multi-strain prebiotics could induce humoral immune response for ND vaccine in broiler chickens.

Histopathological examination was varied from organ to another, as bursal sections in all vaccinated groups showed varied degree of histopathological changes from one vaccine to another as it showed slight lymphoid follicle depletion in groups received monovalent ND-SVRI and monovalent ND Laprovet® (fig. 1) while groups received bivalent ND-AI Volvac® and Volvac® ND Conc. KV showed moderate lymphoid depletion together with hyperactivity of the lymphoid follicle (fig. 2), while group received ND Imopest® showed neutrophilia together with hyperactivity of lymphoid follicle that shows even activated germinal center with presence of lymphoblast and mitotic figures. (fig. 3). All vaccinated groups showed normal spleen and thymus.

There were slight histopathological changes in groups received inactivated vaccines together with prebiotics represented in bursa as it showed light lymphoid follicle depletion (fig. 4) with normal spleen and thymus. ***Awaad et al (2010)*** who recorded scoring of histomorphological changes of major immune

organs revealed lymphocytic hyperplasia and activation in Bursa of Fabricius.

After challenge lesion in bursa of both vaccinated groups and those received vaccine together with prebiotics were more aggressive changes which either sever bursal depletion (fig. 5) or sever bursal depletion with connective tissue formation (fig. 6), spleen showed depletion with congestion of red pulp (fig. 7), there was no pathological changes in thymus.

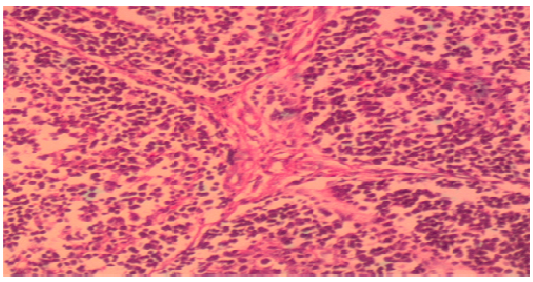

Fig. (1): Bursa of chickens showing moderate depletion of lymphoid follicles (H & E x 66).

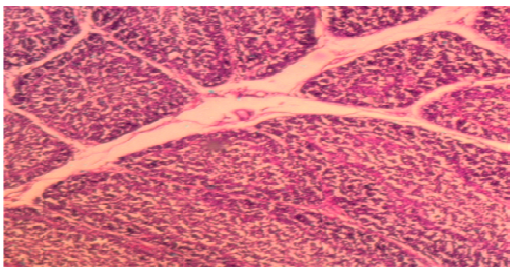

Fig. (2): Bursa of chicken showing lymphoid depletion together with hyperactivity of the lymphoid follicle. (H & E x 33).

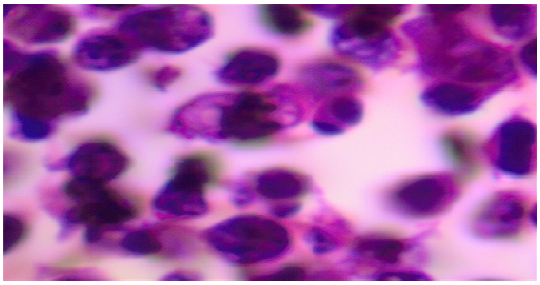

Fig. (3): Bursa of chickens showing activated germinal center with presence of lymphoblast and mitotic figures (H & E x 134).

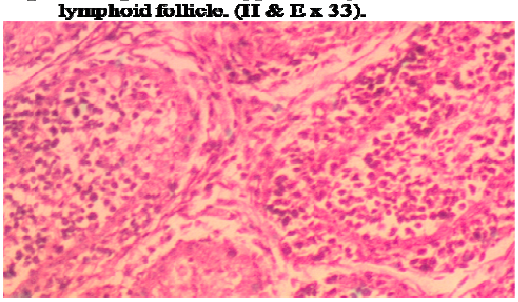

Fig. (4): Bursa of chickens showing slight depletion of lymphoid follicles.(H & E x 66).

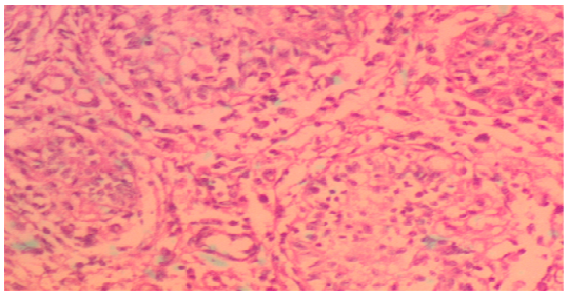

Fig. (5): Bursa of chickens showing severe depletion of lymphoid follicles (H & E x 66).

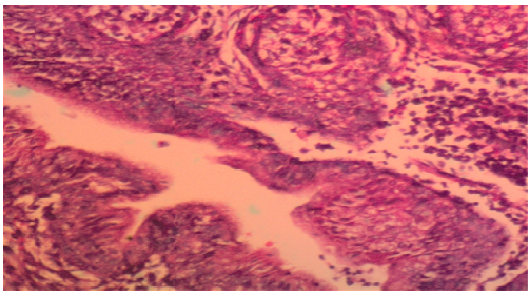

Fig. (6): Bursa of chickens showing sever depletion of the lymphoid follicle with connective tissue proliferation (H & E x 66).

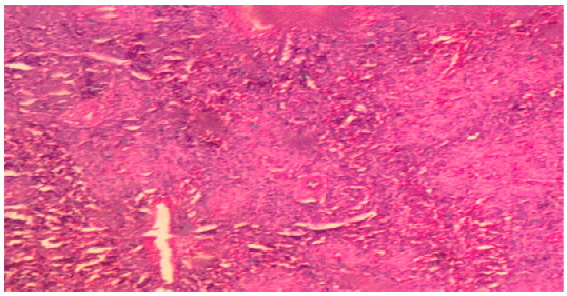

Fig. (7): spleen of chickens showing depletion of the lymphoid follicle with congestion of red bulb (H & E x 33).

In groups received prebiotics alone there is no changes in all immune organs (bursa, spleen and thymus) as it was apparently normal along all experiment time. The alteration between depletion and hyperplasia observed in the current study is considered as a reconstitute strategy.

Effect of virulent virus on control non vaccinated groups were matched with result found by ***Susta et al (2014)*** as they stated that challenge with ND virus induce pathological lesions in lymphoid organs (thymus, spleen, bursa of fabricius, cecal tonsils and gut-associated lymphoid tissues) include extensive lymphoid necrosis.

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