



# **Effect of IBD vaccines on the immunity induced by ND vaccine in broiler chickens**

## **A Thesis Submitted for**

**“Doctor of Philosophy degree in Veterinary Medical Sciences,  
Department of Avian and Rabbit Diseases”**

**Hisham Mohamed Hashim**

(M. D. 2015, Assiut University)

**Under supervision of**

**Prof. Dr. Ragab Sayed Ibrahim**

Professor of Poultry Diseases

Vice Dean for Community Affairs and Environmental  
Development

Faculty of Veterinary Medicine - Assiut University

**Dr. Omar Ahmed kamel**

Assistant Professor and head of Poultry Diseases

Faculty of Veterinary Medicine

Assiut University

**Prof. Dr. Hebat-allah Abdelhalim Mohamed**

Head researcher of Poultry disease

Animal Health Research Institute

Assiut-Lab

**Faculty of Veterinary Medicine**

**Assiut University**

**2021**

<b>Contents</b>	<b>Page No.</b>
<b>List of Contents</b> .....	<b>i</b>
<b>List of Figures</b> .....	<b>ii</b>
<b>List of Tables</b> .....	<b>iv</b>
<b>List of abbreviations</b> .....	<b>vi</b>
<b>Introduction</b> .....	<b>1</b>
<b>Review of literature</b> .....	<b>4</b>
<b>Materials and Methods</b> .....	<b>37</b>
<b>Results</b> .....	<b>51</b>
<b>Discussion</b> .....	<b>86</b>
<b>Conclusion and Recommendations</b> .....	<b>109</b>
<b>Summary</b> .....	<b>111</b>
<b>References</b> .....	<b>115</b>
<b>الملخص العربي</b> .....	<b>--</b>

# List of Figures

<b>NO.</b>	<b>Title</b>	<b>Page No.</b>
<b>1</b>	Average HI titre as detected by HI test at 1-day-old	<b>58</b>
<b>2</b>	IBDV antibody titer distribution in chicken groups using ELISA at 1-day-old.	<b>58</b>
<b>3</b>	Photomicrographs of histopathological changes in bursa of fabricius stained with H&E at the age of 1- day-old	<b>59</b>
<b>4</b>	Average HI titre as detected by HI test at 7-day-old.	<b>61</b>
<b>5</b>	IBDV antibody titer distribution in chicken groups using ELISA at 7-day-old.	<b>61</b>
<b>6</b>	Photomicrographs of histopathological changes in bursa of fabricius stained with H&E at the age of 7- day-old	<b>62</b>
<b>7</b>	Average HI titre as detected by HI test at 14-day-old	<b>65</b>
<b>8</b>	IBDV antibody titer distribution in chicken groups using ELISA at 14 days.	<b>65</b>
<b>9</b>	Photomicrographs of histopathological changes in bursa of fabricius stained with H&E at the age of 14- day-old	<b>66</b>
<b>10</b>	Average HI titre as detected by HI test at 21-day	<b>69</b>
<b>11</b>	IBDV antibody titer distribution in chicken groups using ELISA at 21 days	<b>69</b>
<b>12</b>	Photomicrographs of histopathological changes in bursa of fabricius stained with H&E at the age of 21- day-old	<b>70</b>
<b>13</b>	Average HI titre as detected by HI test at 28-day	<b>73</b>
<b>14</b>	IBDV antibody titer distribution in chicken groups using	<b>73</b>

	ELISA at 28 days	
<b>15</b>	Photomicrographs of histopathological changes in bursa of fabricius stained with H&E at the age of 28- day-old	<b>75</b>
<b>16</b>	Average HI titre as detected by HI test at 35-day	<b>78</b>
<b>17</b>	IBDV antibody titer distribution in chicken groups using ELISA at 35 days	<b>78</b>
<b>18</b>	Photomicrographs of histopathological changes in bursa of fabricius stained with H&E at the age of 35- day-old	<b>80</b>
<b>19</b>	Titer (EID50/ml) distribution in chicken groups using PCR at 3rd day post-challenge	<b>84</b>
<b>20</b>	Titer (EID50/ml) distribution in chicken groups using PCR at 5th day post-challenge	<b>85</b>

# List of Tables

<b>No.</b>	<b>Title</b>	<b>Page No.</b>
<b>1</b>	Commercial vaccines used in the study	<b>39</b>
<b>2</b>	Oligonucleotide Primer. Primers and probes used were supplied from Metabion (Germany).	<b>40</b>
<b>3</b>	Experimental design for the assessment of vaccination program of IBD on the immunity induced by ND vaccine	<b>43</b>
<b>4</b>	Sampling plan	<b>44</b>
<b>5</b>	Preparation of PCR Master Mix for ND real time PCR according to QuantiTect probe RT-PCR kit handbook	<b>49</b>
<b>6</b>	Oligonucleotide Primers and probes. Cycling conditions of Primers and probes used.	<b>50</b>
<b>7</b>	Showing HI titer of different groups at day 1 to day 35 of age.	<b>52</b>
<b>8</b>	BioChek ELISA test showed serological response of broiler chickens with different vaccination program at different time points	<b>54</b>
<b>9</b>	Effect of IBD vaccination on HI titer, ELISA titer and histopathological bursal structure at 1-day-old	<b>57</b>
<b>10</b>	Effect of IBD vaccination on HI titer, ELISA titer and histopathological bursal structure at 7-day-old	<b>60</b>
<b>11</b>	Effect of IBD vaccination on HI titer, ELISA titer and histopathological bursal structure at 14-day-old	<b>64</b>

<b>12</b>	Effect of IBD vaccination on HI titer, ELISA titer and histopathological bursal structure at 21-day-old	<b>68</b>
<b>13</b>	Effect of IBD vaccination on HI titer, ELISA titer and histopathological bursal structure at 28-day-old	<b>72</b>
<b>14</b>	Effect of IBD vaccination on HI titer, ELISA titer and histopathological bursal structure at 35-day-old	<b>77</b>
<b>15</b>	Detection of ND viral shedding by PCR Master Mix at 3 and 5- day post challenged groups	<b>83</b>

# List of abbreviations

IBD	Infectious bursal disease
GD	Gumboro disease
IBDV	Infectious bursal disease virus
HVT	Herpesvirus of turkeys
NDV	Newcastle disease virus
ND	Newcastle disease
APMV-1	Avian paramyxovirus type-1
IFN- $\gamma$	Interferon- $\gamma$
NO	Nitric oxide
BF	Bursa of Fabricius
IL-6	Interleukin-6
GALT	Gut-associated lymphoid tissue
HALT	Head-associated lymphoid tissues
SPF	Specific pathogen-free
CAM	Chorio-allantoic membrane
pi	post-inoculation
RTPCR	Reverse-Transcription Polymerase Chain Reaction
RT	Reverse Transcription
MDA	Maternally derived antibodies
IC	Immune complex
VVND	Viscerotropic velogenic Newcastle disease viruses
NVND	Neurotropic velogenic Newcastle disease viruses
(In-p)	Group vaccinated with intermediate plus

(In/In-p)	Group vaccinated with intermediate- intermediate plus
(In-p/In)	Group vaccinated with intermediate plus - intermediate
Vxx	Group vaccinated with Vaxxitek
Bu-p	Group vaccinated with Bursa-Plex
NC	Negative control
S/C	Subcutaneous
E. D	Eye drop
HA	Hemagglutination
HI	Hemagglutination inhibition
ELISA	Enzyme-linked immunosorbent assay



## **Summary**

Infectious bursal disease (IBD), also known as Gumboro disease, is still a significant threat facing the Egyptian poultry industry. It considers a vital threat to poultry industry worldwide and it has special potentiality in Egypt as the country become endemic. IBD can cause morphologic and histological changes in the BF. Also, cause significant economic losses due to the high mortality and high morbidity resulting from IBDV infection. The immunosuppressive effect of IBDV vaccines is a concern due to the fact that the immunocompromised birds may not demonstrate sufficient titers of antibodies after vaccination against other diseases, such as NDV. For Egypt, ND was ranked as one of the most important poultry diseases in the last decade especially in broiler flocks.

Therefore, prevention is important, and vaccination has become the principal control measure of IBDV infection in chickens. Vaccination of chickens is practiced to provide some degree of protection against disease and it is the main method to control infectious bursal disease (IBD) in commercial broilers worldwide. Broiler have been commonly vaccinated with the recombinant, immunocomplex, intermediate and/or intermediate-plus IBD vaccines to maintain a certain level of immune response to IBDV for prolonged protection. This study was conducted to the effect of IBD vaccines on the immunity induced by Newcastle in broiler and to recommend an effective vaccination program for broilers to suit poultry industry.

In our study, commercial broilers with high levels of MDA were vaccinated with different IBD vaccination programs. A total of 210 chicks were divided into seven groups of 30 chicks each. In Group 1, chicks were

vaccinated with Newcastle vaccine program, Group 2 were vaccinated with intermediate plus live IBD virus vaccine (Avipro Xtreme) at 14 days of age through eye-drop. Group 3 was vaccinated twice with intermediate live IBD vaccine (Avipro Precise) followed by intermediate plus live IBD vaccine (Avipro Xtreme) at 10 and 18 days of age, respectively. Group 4 was vaccinated twice with intermediate plus live IBD vaccine (Avipro Xtreme) followed by intermediate live IBD vaccine (Avipro Precise) at 10 and 18 days of age, respectively, by eye-drop. Group 5 was received the recombinant vaccine (Vaxxitek®) S/C at 1 day of age. Group 6 was received immune complex vaccine (Bursa-Plex®) S/C at 1 day of age. Group 7 was a negative control group, and the chicks did not receive any vaccine.

Blood and bursal samples were collected at 1, 7, 14, 21, 28, 35 days-old chicks randomly to detect the maternal antibody level. Sera are separated and stored at -20 °C until examined. Serum samples were used to determine the antibody titers of ND and IBD. The antibody titers against ND of the birds from various groups were determined by HI and the antibody titers against IBD of the birds from various groups were determined by Biocheck ELISA.

The average of maternal antibody level at 1-day-old is (8011±486.8) for IBD and (8.667± 0.5774) for ND. We found that the maternal antibody titers for IBD in chicks are demonstrable up to 2<sup>nd</sup> week of age which varied between (488.6±23.3) and (852.8±61.34) in all groups. In another hands, group 3 showed lowest level of antibody titre (297.1±35.61).

Most of the vaccination program showed an immunity gap due to the presence of high MDA. Therefore, vaccination at 1 day affected by MDA resulting in the partial neutralization of the vaccine by MDA before the vaccine virus can stimulate the immunity of chickens against IBDV infection.

In the group that vaccinated with intermediate vaccine of IBD (Avipro Precise) at 10 days of age followed by intermediate plus vaccine (Avipro Xtreme), the seroconversion was positive after 21 days-old (2272) and showed a marked increase at 28 days old (6083.5) and 35 days old (9988.2).

The group vaccinated by live intermediate plus vaccine at 10<sup>th</sup> days of age (Avipro Xtreme) and live intermediate vaccine (Avipro Precise) at 18<sup>th</sup> day of age gave a positive ELISA antibody titer starting from 21 days (1829.5), 28 day (9050.5) and 35 days of age (10116.5).

Following vaccination with the recombinant vaccine and immune complex vaccine, a positive IBD ELISA titer detected at 21 days (1150.8), (2235.3) and the titer shows a noticeable increase at 28 (1874.5), (7352.8) and 35 days (3927.8), (6455.8), respectively.

The non-vaccinated control group showed a decrease in maternal antibodies as it did not receive any vaccine. Also, other vaccinated groups showed a gradual drop in maternal antibodies.

The time of vaccination was correlated with the onset of bursa lesions. This was demonstrated in G3 and G4 which showed lymphocytic depletion at the follicular medulla of bursal follicles due to necrosis of lymphocytes from 14 days old.

The laboratory test results and the field immunoassay results demonstrate that the intermediate and/or intermediate-plus vaccine causes a certain degree of bursal damage in broiler chickens but the VAXXITEK® HVT+IBD vaccine causes no damage to the immune organ. So, the major problem with intermediate and/or intermediate-plus vaccines is the induction of lesions in the BF.

Histopathological lesions within the different ages after challenge are varied from minimal lymphocytic depletion at the follicular cortex and medulla in G1, G2 and G5, sever atrophied of bursal follicles in G3

After Challenge of chickens with ND virus  $10^6$ , all the challenged birds in group 7 were died. Protection assessed based on seroconversion, shedding and histopathology. Chickens vaccinated with Newcastle vaccine only were completely protected with no mortality compared with control non vaccinated group that showed (100%) mortality. Viral shedding was disappeared in groups vaccinated with intermediate plus live, Immunocomplex and recombinant vaccine at 5 days post challenge. While viral shedding was reduced in other groups vaccinated with intermediate at 10 days followed by intermediate plus at 18 day and the group vaccinated with intermediate plus at 10 day and intermediate at 18 days.

Moreover, histopathology of G1 and control non vaccinated challenged groups revealed lymphocytic depletion. While G2 and G5 revealed minimal lymphocytic infiltration at the follicular cortex and medulla. Group 3 and 4 showed sever atrophied of bursal follicles.