Evaluation of The efficacy of Purified Egg Yolk Immunoglobulin (IgY) in Preventing and Controlling Newcastle Disease Virus Infection in Broiler Chickens

Fatma M. Radwan¹, Ahmed A. El-Shemy², Mohamed A. Bosila³, Mustafa A. Bastamy⁴, Mohamed M. Amer*¹

¹ MVSc student, Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt.
² Department of Parasitology and Animal Diseases, Veterinary Research Institute, National Research Centre, P.O. 12622, Giza, Egypt
³ Department of Poultry Diseases, Veterinary Research Institute, National Research Centre, P.O. Code 12622 Dokki, Giza, Egypt
⁴ Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, P.O. 12211, Giza, Egypt.

This study evaluated the effectiveness of chicken IgY for the prevention and control of Newcastle disease virus (NDV) genotype VII in vaccinated chickens. The 1/5 and 1/10 diluted IgY had ND titers of 6.87±0.99 and 5.8±0.83, respectively, through subcutaneous (s.c) or intramuscular injections (i.m) of 0.5 ml/bird for 3 successive days. At 21 days of age, after the 3rd injection, NDV-HI titers in prophylactic groups were increased to 6.8±0.89, 5.9±1.14, 5.6±0.83 and 5.5±0.83 in groups given dilution 1/5 and 1/10, respectively. The infected (10⁶ EID₅₀), non-treated birds exhibited symptoms such as respiratory distress, congested comb, greenish diarrhea within 24 hours, and swollen face within 48 hours. The mortality rate was 7.5% by the third-day post-infection. Dead birds were positive for NDV using reverse transcription-polymerase chain reaction (RT-PCR) with a cycle threshold (CT) value 17. 

Chicken received IgY by i.m or s.c, injections, either showed increased HI titers against NDV at 37 days of age after being challenged with the virus. The feed conversion ratio (FCR) at 37 days in the prophylactic groups were 1.81, 1.70, 1.71, and 1.76, respectively. There was no significant difference in FCR between the different IgY dilutions or routes of administration. The prophylactic groups had higher FCR values. The control negative group showed 85.71% mortality within 3-7 days post-infection. The prophylactic and control groups injected with 1/5 showed a 100% protection rate. While groups received 1/10 showed 85.71% and 92.85% for i.m and s.c injections, respectively. RT-PCR analysis of cloacal swabs from treated groups was negative, while nontreated showed positive RT-PCR with CT-17. Groups that received 1/5 diluted IgY showed better results than those that received 1/10 with milder histopathological lesions.

In conclusion: The study demonstrated that the use of chicken IgY against NDV genotype VII in broiler chickens resulted in increased HI titers, mild signs, high protection rates, and FCR. The prophylactic administration of IgY showed better results than control.

Keywords: Chicken IgY, Newcastle disease, Broiler, prophylactic, control, FCR, HI

Introduction

Newcastle disease (ND) is a highly contagious viral avian disease caused by ND virus (NDV), which belongs to the Avulavirus genus of the Paramyxoviridae family [1]. ND poses a significant problem to the poultry industry worldwide and is endemic in many countries [2-4]. It is characterized by respiratory, gastrointestinal, and neurological signs, and with possible high mortality rates in infected poultry. Strict biosecurity measures, good management practices, and vaccination are recommended in preventing and controlling ND outbreaks [4]. However, in areas where NDV genotype VII is prevalent, vaccination may not always be effective [5]. This can be attributed to the interference of maternal antibodies, which can limit the efficacy of vaccination [6].

The immune system of poultry plays a vital role in protecting against pathogenic challenges, and its
proper functioning is mostly associated with poultry health [7]. Egg yolk immunoglobulin (IgY) has gained attention as a preventive and therapeutic agent for various diseases due to its safety, efficacy, and stability [8]. IgY can be produced and purified on a large scale from egg yolk approximately 3 weeks after the last immunization [9-10].

Several studies have demonstrated the protective effect of IgY antibodies against viral infections in poultry. IgY antibodies shown to control and prevent the ND disease [11]. Similarly, IgY antibodies was effectively combating other viral infections such as duck viral hepatitis (DVH) [12], chicken infectious bursal disease (IBD) [13], and influenza virus infections in chickens and ducks [10,14]. IgY antibodies can neutralize viral particles, inhibit viral attachment and entry into host cells, and enhance the immune response against viral infections, thereby providing protection [10,15].

IgY has been suggested as a replacement for the natural generation of conventional polyclonal antibodies in mammals [16]. The supplementation of purified IgY, combined with probiotics, was shown to significantly improve the overall activity of broilers with immune stress in a study conducted by Rehan et al. [17]. This improvement was attributed to a reduction in immune cell count, which is responsible for inflammatory cytokine production and the exaggerated stress experienced during the innate immune response.

IgY is a product derived from serum antibodies synthesized in the blood and transferred to the egg yolk [18-19]. It has gained attention due to its advantages over mammalian IgG, including high yield, low cost, and convenience [20,21]. Additionally, IgY has been reported to have desirable properties such as disease resistance and absence of toxic residues [20,22]. Many specific IgY antibodies have been successfully developed and applied in the prevention and treatment of diseases caused by pathogens [23].

This study aimed to investigate the efficacy of the prepared egg yolk IgY on preventing and controlling NDV infection in experimentally infected broiler chickens.

**Material and Methods**

**Prepared IgY:**

Egg yolk immunoglobulin (IgY) separated and purified by Radwan et al. [10] was used in this study. IgY contained HI antibody titers of 6.87±0.99 and 5.8±0.83 for ND in dilution 1/5 and 1/10, respectively. IgY used as 0.5 ml s.c or i.m injection of dilution 1/5 or 1/10.

**NDV Strains:**

a. **Challenge Virus:** The identified NDV Genotype VIIId isolate with accession numbers MW580389 in GenBank [24] was used for experimental infection of tested groups after propagation and titration in SPF chicken embryos.

b. HI antigen: ND laboratory La Sota strain as allantoic fluid of SPF embryonated chicken eggs was propagated and kept in 2 ml sterile vials at -20 °C. The antigen was adjusted to 4 HA units by HA test before usage in HI test [25].

**Propagation of NDV in Embryonated Chicken Eggs (ECE):**

The used NDV strains were propagated in 9-days-old Embryonated SPF Chicken Eggs [26] For La Sota the allantoic fluid was harvested, cold centrifugation at 3000 rpm for 30 min at 4°C and distributed in Eppendorf tubes. It is measured by slide HA immediately [27]. Calculation EID\(_{50}\) of the challenge infective virus was performed according to Red and Muench [28]. The result of virus titration was10\(^{5.6}\) ml EID\(_{50}\). Chicks were challenged each with 100 ul containing10\(^{8}\) EID\(_{50}\) of NDV through oculo-nasal route [5,24].

**Hemagglutination (HA) test:**

The test procedure was described by OIE [29]. Equal volume of a 1 % (v/v) red blood cells were mixed with 2-fold serial dilutions of allantoic fluid in PBS in a V bottomed 96-well micro-titer plate.

**Hemagglutination inhibitions (HI) test:**

Two-fold serially diluted samples with pbs were incubated with equal volume containing 4 HA unite viruses in V-bottom 96-well microtiter plates at 37°C for 30 minutes. At the end of incubation, freshly prepared 1% chicken red blood cells (CRBC) were added, and plates were mixed by agitation, covered, and allowed to set for 30 minutes at room temperature. The HI titers were determined by the reciprocal of the last dilution which contained non-agglutinated CRBC. Positive and negative control samples were included on each plate [13,30].

**Experimental chicks:**

One hundred and sixty (160) one-day- broiler Hubbard chicks as hatched were kindly supplied by Cairo 3A poultry Company.

**Ration:**

The experimental chicks reared in experimental ages department of poultry diseases, faculty of veterinary medicine, Cairo university, and fed on with Cairo 3A ration including starter ration (23% protein), grower ration (21% protein) and finisher ration (19% protein) according to Hubbard breed manual.
Vaccination:

The chicks were vaccinated against ND and infectious bronchitis by Izovac H120-B1 Hitchner (batch NO:3245 and Expire date 7/2024) at 8th day from age by eye drop method. Trivalent vaccine against HPAI H5N8, H5N1 and ND in activated at the 10th day of age (Batch No:2209010101 & Expire date 9/2024). Live attenuated vaccine against Infectious Bursal disease (Mevac IBD 818 with batch No 2205150401 & Expire date 5/2024) at the 14th day, while the live ND vaccine (Polimune La Sota strain batch No: 1362 &Expire date 2/2024) was given through eye drop at the 18th day of age.

RT-PCR for detection of NDV:

One step RT-PCR was carried using QIAGEN® One Step RT-PCR kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions. RT-PCR was used for the detection NDV using the following primers: NDV Forword primer (25 bp) 5'-AGTGATGTCGGGACCTTC-3' and Reverse primer (20bp) 5'-CCTGAGGAGAGCATTCTGCCATGCA3', NDV prob 5'-Fam-AGTGATGTGCTCGGACCTTC-BHQ3'. Thermal cycling RT one at 15 min at 55˚ C, Polymerase activation 1 min at 95 ºC, denaturation 40 cycle for 10 sec at 95 ºC and Annealing for 30 sec at 60 ºC.

Blood samples for sera:

Blood samples (20) were collected at 1 day as well as 10 samples/group just before challenge and at the end of observation period at age of 21 and 37 days of age: respectively. Sera were separated and subjected to HI test against ND antigen to detect Hemagglutinating inhibiting (HI) antibody titers. All blood samples were centrifuged at 3500 rpm for 15 minutes for serum. Sera was treated with heat for 10% formol saline and fixed on 10% formol saline and subjected for histopathological examination. Samples were prepared for stain by H&E. section covered with slides and examined by light microscope [32].

Monitoring of shedding of virus by collecting cloacal swab:

At the 7th day post challenge (age of 28 days, the 8th day from 3rd prophylactic dose, and 2nd day from 3rd treatment dose) and at the 37th day (16 day from 3rd prophylactic dose and 11th from 3rd treatment dose),3 cloacal swabs were collected from each group and pooling and tested for the presence of challenging NDV using RT-PCR for detection of virus shedding.

Experimental design:

At the 18th day of age, the used 160chicks were divided into 2 mean groups A and B, each 80 birds (Table 1). Group A was further divided to be control negative (group 1) and prophylactic groups (2- 5), 16 birds each. Groups 3-6 were injected with IgY 3 successive times at the 18,19, and 20th day of age. At the 21st day of age all groups were infected each with 100 ul containing 10^6 EID50 of challenge NDV genotype VII. At the age of 25 days after appearance clinical signs, mortality, and post-mortem lesions as well as confirmation of positive mortality by RT-PCR. The remaining birds of apparent healthy chickens (70) of the mean group B was further divided into 5 groups to be positive control (6) and treatment groups 7-10, 14 birds each. Birds of groups 7-10 were 3 times injected with IgY at 25,26 and 27 days. Three cloacal swabs were collected from each group at 28 and 37 days of age for RT-PCR. AI groups were subjected to daily observation for signs, mortality with record of postmortem lesions. Two chickens /group at the 10th day post challenge were sacrificed for collection of tissue samples for histopathological examination. Weekly feed intake and body weight gain were recorded.

Histopathological examination

Two chickens from each group were slaughtered at day 6th post ND challenge for histopathological examination, affected organs including the thymus, spleen, cecal tonsils and bursa were collected and fixed on 10% formol saline and subjected for histopathological examination. Samples were prepared for stain by H&E. section covered with slides and examined by light microscope [32].

Statistical analysis

The data are represented as mean ± SD. Statistical analysis started by validating the assumptions of normal distribution and homogeneity of variance. Then, differences in IgY and control negative were tested using independent sample t-test. p-values < 0.05 were considered statistically significant.

Results and discussion

Newcastle disease is a significant concern for the poultry industry globally, and strict biosecurity measures, good management practices, and vaccination are essential for its control. However, the efficacy of vaccination can be limited in the presence of maternal antibodies [5,33]. IgY antibodies can be produced on a large scale and have shown promise in preventing and controlling viral infections in poultry, including ND and AI [8-10].

At the day one, the used commercial chicks have 4.11±1.65 HI mean titer of maternally derived antibody against NDV. The used vaccines against NDV were resulted in HI titers of 3.78± 2.53 at the 21st day of life (Table 2). Similar results were reported by Ahmed et al. [24], Amer et al. [34] and Ahmed et al [35], where similar vaccines and timing were used in commercial broiler chickens.
TABLE 1. Chicken groups at the 18th day of age, aim, and route of diluted IgY injection

<table>
<thead>
<tr>
<th>Mean group</th>
<th>Sub groups</th>
<th>Aim</th>
<th>No of chicken</th>
<th>Treatment</th>
<th>Route of injection</th>
<th>Challenge at 21days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Prevention</td>
<td>16</td>
<td>Negative control</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/5 i.m</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/5 s.c</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/10 i.m</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/10 s.c</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Prevention</td>
<td>14</td>
<td>Positive control</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>B*</td>
<td>7</td>
<td></td>
<td>14</td>
<td>IgY 1/5 i.m</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Control</td>
<td>14</td>
<td>IgY 1/5 s.c</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Control</td>
<td>14</td>
<td>IgY 1/10 i.m</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Control</td>
<td>14</td>
<td>IgY 1/10 s.c</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* Out of the 80 chickens in this group 6 were died and 4 illing was rejected and the rest 70 were used.

TABLE 2. Result of mean HI titers against VDV in serum of controls, prophylactic and control infection group (n=10)

<table>
<thead>
<tr>
<th>Mean group</th>
<th>Sub group</th>
<th>Aim</th>
<th>No of chicken</th>
<th>Treatment</th>
<th>Route of injection</th>
<th>Age of birds/days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Prevention</td>
<td>16</td>
<td>Negative control</td>
<td>-</td>
<td>3.78± 2.53</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/5 i.m</td>
<td>+</td>
<td>6.8± 0.89</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/5 s.c</td>
<td>+</td>
<td>5.9±1.14</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/10 i.m</td>
<td>+</td>
<td>5.6±0.83</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Prevention</td>
<td>16</td>
<td>IgY 1/10 s.c</td>
<td>+</td>
<td>5.5±0.83</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Prevention</td>
<td>14</td>
<td>Positive control</td>
<td>+</td>
<td>3.78±2.53</td>
</tr>
<tr>
<td>B*</td>
<td>7</td>
<td></td>
<td>14</td>
<td>IgY 1/5 i.m</td>
<td>+</td>
<td>2.25±2.09</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Control</td>
<td>14</td>
<td>IgY 1/5 s.c</td>
<td>+</td>
<td>3.21±2.00</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Control</td>
<td>14</td>
<td>IgY 1/10 i.m</td>
<td>+</td>
<td>2.27±1.90</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Control</td>
<td>14</td>
<td>IgY 1/10 s.c</td>
<td>+</td>
<td>3.23±2.09</td>
</tr>
</tbody>
</table>

* Significant at p < 0.05. SD: Stander deviation. ND*: not done.

At 21 days of age and after the 3rd injection with IgY the detected HI titers against NDV were significant higher (p < 0.05) than control group 1, where titer in 1/5 dilution (Gr 2 and 3) was 6.8± 0.89 and 5.9±1.14, in dilution 1/10 (Gr 4 and 5) was 5.6± 0.83 and 5.5± 0.83 with injection in i.m and s.c, respectively (table 2). Administration of IgY increases the antibody level in face of infection [16].

The non-injected groups 6-10 showed HI titer ranged from 2.15± 1.56 to 3.21± 2.00 as result of immune response to administered vaccines. These findings were previously cited by [5,24,35] and also, broiler chickens with ND maternal antibody does not respond good to vaccination as Niewiesk [36] stated that although the MDAbs has no protective titer against infection but still inhibit vaccination against infectious diseases of humans and animals.

The detected HI antibody levels in IgY injected chickens either i.m or sic s.c and recovered challenge at 37 days of age showed increased HI titers to be 4.2± 0.83 to 4.4±1.14 in prevention groups as well as 3.8± 0.83 to 4.2±1.0 in control groups (Table 2). while lower titer in control negative (3.8± 0.83) was still detected. Usually, HI titers increased in recovered birds 7-10 days post infection and reach the maximum after 21 days from the last vaccination then gradually decline [37].

The infected non treated bird mean group B showed off food respiratory sound congested comb and greenish diarrhea were seen 24 hours post infection while respiratory signs were noticed in 48 hs with swollen face (Fig 2). At the 3-day post infection 6/80 birds were died from group B at rate of 7.5%. Tissue samples from dead birds including...
lung, liver and spleen were subjected to viral RNA extraction followed by RT-PCR showed positive results with CT17 against positive test control that showed CT 15.

It was noticed that FCR of all birds was 1.71 at 15 days of age (Table, 3). FCR of the control negative group 1 was the best (1.47, 1.43, and 1.55) in 25, 28 and 37 days of life, respectively, while infected non medicated group 6 was the lowest (1.84 and 1.91) at 25 and 28 days of live.

FCR (1.81, 1.70, 1.71, and 1.76), respectively, without marked difference between the used IgY dilution or rout of administration. control groups 7, 8, 9, and 10 at the 37th day showed.

Fig. 1. Result of RT-PCR on extracted viral RNA against primers for detection of NDV RNA give positive results at CT 17 and Positive control at CT 15

The lowest FCR (1.85, 2.03, 2.27 and 2.05) (Table 3). Groups of birds given IgY by i.m showed FCR better than those given IgY by s.c injection. Groups injected with IgY before infection (IgY prophylactic groups) showed higher FCR than those the infected followed by injection after appearance of signs and mortality (IgY control) treated groups. The results indicated that NDV challenge resulted in lower FCR [24, 38]. IgY administration as prophylactic resulted in mild signs and short disease course without mortality that reflected on the FCR [10]. The calculated broiler FCR as a guide for performance and on the optimization of flock condition [39,40]. Control non medicated positive group (Gr 6) showed mortality at the 3rd day post infection. by 3-7 days till mortality reach 12/14 birds (85.71% ) and only 2 birds out of 14 were protected (14.29%) due to the used vaccines.

Fig. 2. Swollen head in positive control. Fig. 3. Hemorrhage on tips of proventriculus glands.
TABLE 3. Feed conversion rate of given diluted IgY i.m or s.c before and after challenge with NDV genotype VII.

<table>
<thead>
<tr>
<th>Mean group</th>
<th>Sub groups</th>
<th>Aim</th>
<th>No of chicken</th>
<th>Treatment</th>
<th>Route of injection</th>
<th>Age of birds/days</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Prevention</td>
<td>16</td>
<td>Negative control</td>
<td></td>
<td>1.47 1.43 1.55</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>16</td>
<td>IgY 1/5</td>
<td>i.m</td>
<td>1.66 1.79 1.81</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>16</td>
<td>IgY 1/5</td>
<td>s.c</td>
<td>1.66 1.62 1.70</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>16</td>
<td>IgY1/10</td>
<td>i.m</td>
<td>1.55 1.59 1.71</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>16</td>
<td>IgY 1/10</td>
<td>s.c</td>
<td>1.71 1.54 1.74 1.76</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>14</td>
<td>Positive control</td>
<td></td>
<td>1.84 1.91 ND*</td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>Control</td>
<td>14</td>
<td>IgY 1/5</td>
<td>i.m</td>
<td>1.81 1.72 1.85</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>14</td>
<td>IgY 1/5</td>
<td>s.c</td>
<td>1.79 1.83 2.03*</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td>14</td>
<td>IgY1/10</td>
<td>i.m</td>
<td>1.73 1.69 2.27*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>14</td>
<td>IgY 1/10</td>
<td>s.c</td>
<td>1.52 1.59 2.05*</td>
</tr>
</tbody>
</table>

ND*: not done. *Significant p-values < 0.05

TABLE 4. Result of challenge with NDV genotype VII in IgY injected broiler chicken and protection rat

<table>
<thead>
<tr>
<th>Mean group</th>
<th>Sub groups</th>
<th>Aim</th>
<th>Treatment</th>
<th>Route of injection</th>
<th>No of chicken</th>
<th>No of dead</th>
<th>No of Live</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Prevention</td>
<td>Negative control</td>
<td></td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>IgY 1/5</td>
<td>i.m</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td>IgY 1/5</td>
<td>s.c</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>IgY1/10</td>
<td>i.m</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>IgY1/10</td>
<td>s.c</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>Positive control</td>
<td></td>
<td>14</td>
<td>12</td>
<td>2</td>
<td>14.28*</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
<td>IgY 1/5</td>
<td>i.m</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>Control</td>
<td>IgY 1/5</td>
<td>s.c</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td>IgY1/10</td>
<td>i.m</td>
<td>14</td>
<td>1</td>
<td>13</td>
<td>85.71*</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>IgY 1/10</td>
<td>s.c</td>
<td>14</td>
<td>2</td>
<td>13</td>
<td>92.85</td>
</tr>
</tbody>
</table>

*Significant p-values < 0.05

It was reported that NDV sub-genotype VII was predominant in Egypt from 2012 and still considered the predominant strain that induce several outbreaks in poultry with high mortality in most of chicken farms in spite of the intensive vaccination programs including both live and killed NDV genotype II vaccines [41-43].

On the other hand, prophylactic treated groups either s.c or i.m infected showed no mediated signs only lower in feed in the first 24 hours. In control medicated groups the detected signs disappeared at the 48 hours with the 2nd injection in both s.c and i.m groups. Groups with 1/5 diluted IgY showed better results than those given 1/10.

Generally, no mortality was recorded in all prophylactic groups (3-5), where the protection rate was 100%. The groups 6-10 those received IgY for control of the challenge showed a protection rate of 100%, 100% in 1/5 injected groups. The 1/10 injected groups 9 and 10 showing protection rate 85.71% and 92.85% for i.m and s.c group, respectively (Table 4). This result was previously
recorded by Wallach et al. [44] who reported that in vivo studies in a mouse model showed that intranasal administration of H5N1-specific IgYs 1 hour prior to infection had a 100% protection against lethal challenge with H5N1. Also, Wills et al. [45] found that s.c administration of egg yolk containing high levels of specific ND IgY antibody protected 80% of the birds during a four-week study period. Intranasal administration of H5N1-specific IgYs in mice before and after lethal infection with H5N1 and H5N2 resulted in complete recovery of the infection [14]. The aim of IgY is to rise the HI titer in chicken before or after ND infection as it was reported that there is an inverse relationship between chicken mortality and mean HI titer and Chickens with higher ND HI antibody titers had better survival rate to the challenge [46].

Result of RT-PCR on extracted viral RNA against primers for detection of NDV RNA from cloacal swabs at 28 days of age (8th day from 3rd prophylactic dose, and 2nd day from 3rd treatment dose) and at the 37th day (16 day from 3rd prophylactic dose and 11th from 3rd treatment dose) give negative result in treated groups either in prevention or control. While as well as the control negative (noninfected non treated group). Similar results were reported by Lee [47] found that IgY results in reduction inH1N1 viral and damage to the lung tissue in mice. Moreover, whole-virus-based IgY lowered the levels of virus shedding and prevented mortality [48]. On the other hand, the control positive give positive result at CT 25 and positive control of virus at CT 15 at the 8th day of infection [49].

Histopathological examination of control negative tissue sections (group 1) showed normal structure. Positive control (Group 6) tissue sections reveal hemorrhages in liver (Fig 4a), submucosal proventriculus hemorrhages (Fig 4b), massive tracheal submucosal congestion accompanied with hyper activity of the goblet cells in the mucosa (Fig 4c), to moderate congestion of tunica mucososa (Fig 4d). Moreover, Spleen showed area of hemorrhages and depletion of the lymphoid follicles (Fig 4e) and Submucosal hemorrhages in trachea with lymphocytic infiltration (Fig 4f). While the spleen showed spleen depletion (Fig 4g). The recorded lesion was previously recorded by several researchers (Amer et al. 2018, Ahmed et al, 2022a). Grs 2-5 those received IgY in dilution 1/5 and 1/10 via i.m and s.c showed tracheal hemorrhage (Fig 4a), liver congested portal vein (Fig 4e), proventriculus congestion (Fig 4f), spleen hemorrhage (Fig 5a), tracheal inflammation (Fig 5b) and tracheal hemorrhages (Fig 4d). Gropes 7-8 those received IgY in dilution 1/5 and 1/10 via i.m and s.c for control of NDV infection showed histological changes including hemorrhages in cecal tonsil (fig 6a), and proventriculus lymphocytic infiltration (Fig 6b), moderate liver congestion (Fig 6c) and liver necrosis (Fig 6d). The spleen and Trachea showed no lesion in all IgY treated groups and lesions were seen in digestive tract. The potential therapeutic applications of chicken specific IgY in the prevention and treatment of respiratory infections had been detected and described, with a remarkable activity in neutralizing the pathogen in both the respiratory tract and lungs had been [10,16]. The recorded lesion was less severe in birds that received IgY at dilution of 1/5, it was found that IgY was shown to improve drug efficacy by reducing ulcer lesions [50], while route of injection shows negligible role in lesions. Recovery from signs was dependent on dose and repetition of chicken IgY administration [51], also in prevention of bacterial and viral infections [52,53].
Fig. 4. Histopathological lesion in tissue section of chicken group infected with NDV genotype VII (H&E):

a. Severe congestion of the central vein in liver (arrows) (x100).
b. Massive submucosal proventriculus hemorrhages (Arrows) (X 100)
c. Tracheal showed submucosal congestion accompanied with hyperactivity of the goblet cells in the mucosa (Arrows) (X100)
d. Tracheal suffering from congestion of tunica muscosa (Arrows) (X100)
f. Submucosal hemorrhages in trachea with lymphocytic infiltration (Arrows) (X200)
e. Spleen showed area of hemorrhages and depletion of the lymphoid follicles (Arrows) (X100)

Fig. 5. Histopathological lesion in tissue section of chicken groups received IgY prophylactic of NDV genotype VII infection (H&E):

a. Spleen suffering from congestion of the red bulb (Arrows) (X100). b. Tracheal showed inflammatory cells infiltration of the mucosa accompanied with severe hyperactivity of the goblet cells in-between (X100).
Fig. 6. Histopathological lesion in tissue section of chicken groups infected with NDV genotype VII, and received IgY for control of infection (H&E):

a. Severe congestion in cecal tonsil (Arrows)(X100).
b. Proventriculus with severe lymphocytic infiltration of the mucosa (Arrows)(X100).
c. Liver showed an area of coagulative necrosis infiltrated with lymphocytes (Arow)(X200).
d. Severe congestion of the central vein (Arow)(X100).

Conclusion and recommendation

In the present study, the prepared egg yolk IgY investigated its effect on controlling and preventing NDV infection in broiler chickens. The use of IgY as a passive immunization strategy has shown promise as an alternative approach to combat the prevalent and the new emergence of pathogens.

Ethics approval and consent to participate:


Acknowledgments

The authors thank the Department of Poultry Diseases, Faculty Veterinary Medicine, Cairo University, Parasitology and animal disease Department, Veterinary Research Division, National Research Centre, Giza. Egypt, and Egyptian laboratory for poultry health, Bader Center, El-Behera. for facilities during this study. This work was self-funded by team members and all authors declared that they did not receive any specific fund for this study.

Authors’ contributions

Fatma M. Radwan and Ahmed Ali El-Shemy collected samples and carried out laboratory and field work. Mohamed A. Bosila carried out the histopathological examination. Mostafa A. Bastamy and Mohamed M. Amer supervised the manuscript. All members revised the original draft and approved the final manuscript.

Data availability

The authors confirm that the data supporting the findings of this study are original, resulted from experimental work and available within the article [and/or] its supplementary materials.

Funding statements

This work was done by author’s activity without any fund.

References


42. Saad, A.M., Samy, A., Soliman, M.A., Arafa, A., Zanaty, A., Hassan, M.K. and Hussein, A.H. Genotypic and pathogenic characterization of genotype VII Newcastle disease viruses isolated from commercial farms in Egypt and evaluation of...


تقييم فعالية الجلوبولين المناعي لصفار البيض المنقى (IgY) في الوقاية والسيطرة على عدوى فيروس مرض النيوكاسل في الدجاج.

قاطنة محمد رضوان، أحمد علي الشيمي، محمد عبد الرحمن بصيله بوصيلة، مصطفى أحمد بطماني، محمد محرس عامر.

أجريت هذه الدراسة تقييم فعالية IgY في الدجاج والأسان المعزول (NDV) NDV هو فيروس خاص بالدجاج، وهو فيروس يحتوي على NDV (IgY) البالغ 45290393 10.1016/0022-1208(93)90204-3. استخدم الممر التخفيفات NDV-HI أو مضاد يحتوي على NDV-HI على الأضحى. بعد الحقنة الثالثة لطائف أو تخفيف، نتائج أفضل من تلك التي تلقت المجموعة الضابطة. أظهرت جميع المجموعات ومجموعة الضابطة التي تم حقنها ب-IgY، نسبة تحويل الغذاء FCR التي تلقت IgY قبل الإصابة، أعلى من FCR المجموعة الضابطة بـ 0.89 ± 5.83 إلى 1.14 ± 5.93 وإلى 6.8 ± 5.53. بعد ظهور الأعراض ووفيات في المجموعة السلبية، كان معدل الوفيات FCR لمجموعة الضابطة 1.76 ± 0.83 إلى 4.4 ± 3.83. وفي المجموعة الضابطة، نسبة تحويل الغذاء FCR لمجموعة الضابطة تم تقييم نسبة تحويل الغذاء FCR عند عمر 7.5 يومًا، ونسبة تحويل الغذاء FCR في القسم الضابطة 3.8 ± 0.83 إلى 4.2 ± 0.83. وكانت المجموعة الضابطة نجحت في الحماية. كانت المجموعات التي تلقت IgY للفيروسات NDV VII وقد دث أظهر العلاج الوقائي على التوالي. لم يكن هناك اختلاف كبير في FCR بعد ظهور الأعراض ووفيات في المجموعة الضابطة. نشأت الفيروسات NDV VII وانية، السيطرة.

الكلمات الدالة: IgY في الدجاج، مرض نيوكاسل، الدجاج، المنع، المناعي، السيطرة.