SEROLOGICAL STUDIES ON THE EFFECT OF ROUTE OF VACCINATION OF NDV VACCINE ON THE IMMUNE RESPONSE OF CHICKENS

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Abstract

The present work aimed to study the effect of different routes of vaccination (Ocular – Per Os – Intramuscular – Intranasal and Spraying) against NDV on the immune response of vaccinated chickens. The hemagglutination inhibition (HI) test and enzyme-linked immunosorbent assay (ELISA) technique were performed.

The challenge test of birds given ND viral vaccine showed the following: Protection rates of 82.35 %, 72.2 %, 66.7 %, 63.16 %, 56.22 % and 27.2 % for birds vaccinated by O, I/M, I/N, spraying and control group, respectively. Results reveled that for Newcastle disease virus, ocular route of vaccination is the best one, then per os and intra-muscular routes (favourable), and intranasal and spraying routes are unfavourable and not recommended.

INTRODUCTION

NDV was firstly identified by Doyle, (1927) and it is classified as paramyxo virus by Andrews and Pereira (1972), and Lancaster and Alexander (1975), during the period from 1957 – 1963. Sheble (1963)revealed 38 isolates all of which were velogenic viscerotropic and neurotropic strains in Egypt. Khafagy (1983) identified 17 velogenic strains and 3 lentogenic strains, Amal Eid (1988) revealed 15 velogenic strains of NDV in Sharkia province.

Alexander and Allan (1973) suggested that NDV strains vary considerably in their biological properties including their virulence to chickens. Sigmann and Woernle (1955) subjected monospecific antisera prepared against 9 different

strains of NDV to HI test. Hemagglutination inhibition test used to measure the immune response of chickens because it is simpler and faster than virus neutralization test (Brandly *et al.,* 1947 and Hanson 1980), the first vaccination applied at 7 days old chicks according to Jusko *et al.* (1964) who reported that gamma-globulin and specific antibodies appeared on the 6th day in the serum of chickens .

Cadman *et al.* (1997) compared between hemagglutination inhibition (HI) test and enzyme linked immunosorbent assay technique. They stated that there is segmoidal relationship (r=0.62, 3rd degree polynomial) between antibody level detected by HI and ELISA .

MATERIALS AND METHODS

A- Material

1 - Chicks: 240 one day old chicks were used for NDV vaccination and the chicks also reared in hygienic isolated cages, fed commercial ration and clean water.

2 - Embryonated chicken eggs : Fertile chicken eggs were incubated at 37 ^{o}C till 11 days used for both virus propagation and titeration.

3 - Newcastle disease virus vaccine : ND viral vaccine (B-1 type, Live virus, LaSota strain) was obtained from CAEVA Company. The vaccine was preserved at 4 ^{o}C till used. The producer advised to be given by any route.

4 - Newcastle disease virus (virulent strain): Velogenic Viscerotropic Newcastle disease virus obtained from Newcastle disease Production and Research Institute, Abbasia, Cairo, Egypt. It was used for the challenge test of vaccinated and non vaccinated birds. The titer of the virus was Log_{10} 8 EID_{50} / ml. Very virulent Newcastle disease virus was preserved at –20 ^{o}C till used.

5 - Biological reagents

- Hanks balanced salt solution (HBSS)

It was prepared according to Hanks and wallace (1949).

- Sodium bicarbonate solution

4.4% bicarbonate solution in de-ionized distilled water was used for adjustment of the required pH of the cell culture media and other media.

- Antibiotic stock solution

One vial of penicillin-G-sodium (million units) and one vial of dihydrostreptomycin sulphate (one gram) were dissolved in 100 ml phosphate buffer saline (PBS) . It was added to Hanks medium at a final conc. of 100 i.u. penicillin and 100mg streptomycin per each ml of medium.

- Physiological saline

8.5 g NaCl were dissolved in 1000ml distilled water , sterilized by autoclaving and used for washing chickens R.B.Cs.

- Chicken erythrocyte suspension

R.B.Cs were collected by vein puncture in 4% sodium citrate as anticoagulant. The R.B.Cs were washed 3 times with physiological saline by centrifugation at 800 rpm for 10 minutes. Washed R.B.Cs were diluted to make a 0.5% suspension in saline.

Nutrient agar, tetrathionate broth, MacConkey agar, nutrient agar, Sabouroud `s agar and PPLO media (Difco) were used for sterility tests.

- Reagents for ELISA

- Coating buffer
- Phosphate buffer
- Washing buffer
- Blocking buffer (PBS + Bovine albumin)
- Substrate buffer (pH 5.0)
  - Citric acid phosphate buffer
  - Chromogenic substrate
- Stopping solution (1.25 M. H2So4)

B- Methods

1 - NDV propagation in embryonated chicken eggs (ECE)

- It was carried out according to Anon (1990 a)

2 - NDV titration in embryonated chicken eggs

- It was carried out according to Read and Frunch (1983).

3 -Vaccination Schedule

The optimum time of vaccination against NDV was determined at the age of 8 days old. We classified the chicks into six groups (each group contain 40 chickens) to be vaccinated by various routes as follows:

I- First group vaccinated ocularly (O).

II- Second group vaccinated intranasaley (I/N).

III- Third group vaccinated per os (P/O).

IV- Fourth group vaccinated by Spraying.

V- Fifth group vaccinated intramuscularly (I/M).

VI- Six group was non-vaccinated (Control).

- We prevented the use of antibiotics 48 hours in drinking water before vaccination and prevented drinking of water 3 hours before vaccination.
- Then vaccination by CAEVA company vaccine (B-1 type, LaSota strain) was carried out, the dose of virus is $\log_{10}$ 6.8 EID_{50}/ml, then another dose of the same vaccine (boostering dose) was given on the 18^{th} day.
- At age of 44 days old, we carried the challenge test using virulent strain of Newcastle Disease Virus (NDV) (titer of virus $\log_{10}$ 8 EID_{50}/ml).
- Birds are put under observation for 15 days post-challenge, then symptoms and dead birds recorded and protection percent calculated.

4 - Sampling

Serum Samples

- Blood samples were collected by heart puncture pre-vaccination as well as post-vaccination at predetermined intervals. Collected blood was allowed to clot in a slope position and separated by centrifugation at 800 rpm for 10 minutes, and collected sera stored at –20°C till used .

5 - Serological test

Rapid plate hemagglutination test

- It was applied according to the standard method detailed in (Methods for examination of poultry biologics and for identifying and quantifying avian pathogen, 1971).

Hemagglutination Inhibition

- The test was carried out according to Giambrone (1979).

Procedures of serum preparation and separation for testing the prepared ELISA antigen plates specific for NDV

- The test was carried out according to Agri-check kits.

Statistical Oanalysis

The results obtained were statistically analysed according to Snedecor (1969).

RESULTS

Table 1. Results of HI test and ELISA technique on sera samples randomly collected from one-day chicks.

No.of sera samples	Result of HI* test titer	Result of ELISA** titer
1	128	508.2
2	128	827.6
3	64	1728.3
4	64	1524.5
5	32	602.8
Mean	83.2 ±19.2	1038.28 ±64.2

HI* : Hemagglutintion inhibition.
ELISA** : Enzyme linked immunosorbent assay.

Table 2. Results of HI test and ELISA technique on sera samples randomly collected from 7-days chicks.

No.of sera samples	Result of HI* test titer	Result of ELISA** titer
1	32	945.9
2	16	360.6
3	32	452.5
4	16	344.2
5	16	206.8
Mean	22.4 ±3.2	462 ±93.2

HI*: Hemagglutintion inhibition.
ELISA** : Enzyme linked immunosorbent assay.

Table 3. Results of mean HI Titers at various intervals (per days) following vaccination with NDV vaccine by various routes as well as post-challenge (15 days) with V.V.N.D.

ROUTE OF vaccination	Results of mean HI* titer at various intervals post-vaccination by various routes (per day).			
	18	30	42	60***
O	83.2±19.2	224±85.86	179.2 ± 84.2	332.8 ± 65.80
Per Os	80± 44.6	198.4± 87.2	140.8± 31.35	217.6 ± 79.93
I/M	64± 44.6	166.4± 39.4	84.8 ± 44.08	204 ± 84.42
I/N	49.6±21.8	142.4± 94.6	96 ± 20.24	102.4 ± 42.21
Spraying	54.4 ± 19.9	121.6± 38.4	89.6± 15.68	59.2 ± 20.17
Control	27.2 ± 9.9	27.2 ± 9.9	25.6 ± 10.55	28.8 ± 10.31

HI*: Hemagglutintion inhibition.

60***: 16 days post-challenge.

Table 4. Results of mean ELISA Titers at various intervals (per days) following vaccination with NDV vaccine by various routes as well as post-challenge (15 days) with V.V.N.D.

ROUTE OF vaccination	Results of mean ELISA** titer at various intervals post-vaccination by various routes (per day).			
	18	30	42	60***
O	563.4 ±89.6	794 ± 105.86	876.2±114.2	1340.8±06.5
Per Os	486 ±64.5	688.3 ± 97.6	590.6±92.3	921.6±109.9
I/M	554.3± 84.5	665.4 ±99.2	714.8±84.08	904±94.42
I/N	400.8±92.6	742.3±104.6	700.5±80.92	878.43±102.6
Spraying	452.4±90.9	712.4±88.3	682.4±95.6	755.2±120.2
Control	325.2±79.9	479.4±90.96	409.5±110.5	All birds are dead

ELISA** : Enzyme linked immunosorbent assay.

60***: 16 days post-challenge.

Table 5. Protection percentage of vaccinated birds challenged with NDV at 16 days post-challenge for each group according to route of vaccination.

Route of vaccination	Total No. of Challenged birds	Dead birds at 15th day post-challenge	protection percent (%)
O	17	3	82.35
P/O	18	5	72.2
I/M	18	6	66.7
I/N	19	7	63.15
Spraying	16	9	56.22
Control	18	13	27.8

DISCUSSION

Pre-vaccinated sera samples from chicks used for ND vaccination contained a mean HI titer of 83.2 and mean ELISA antibody titer of 1038.28 at age of one day old chicks then started to decline till reaching a mean HI antibody titer of 22.4 and mean ELISA antibody titer of 462 just before vaccination (on 7 days old).

Vaccination by ocular route resulted in an immune response with hemagglutinating inhibiting antibodies peak titer 224 at 22 days post vaccination (30 days old chickens) and ELISA antibody peak titer of 1340.8 on 60 days old chickens (15 days post-challenge). Birds vaccinated per os revealed an immune response with HI antibody peak titer (198.4) on 22 days post-vaccination (30 days old chickens) and peak ELISA antibody titer of 921.6 on 60 days old chickens (15 days post-challenge).

Birds vaccinated intramuscularly revealed an immune response with HI antibody titer (166.4) on 22 days post-vaccination (30 days old chickens) and peak ELISA antibody titer of 904 on 60 days old chickens (15 days post-challenge).

Birds vaccinated intra-nasally showed an immune response with HI antibody peak titer (142.4) on 22 days post-vaccination (30 days old chickens) and peak ELISA antibody titer of 878.43 on 60 days old chickens (15 days post-challenge).

Birds vaccinated by spraying route revealed an immune response with HI antibody peak titers (121.6) on 22 days post-vaccination (30 days old chickens) and peak ELISA antibody titer of 755.2 on 60 days old chickens (15 days post-challenge).

Then the HI antibody titers started to decrease but with still titers above 64 for groups vaccinated ocularly, per os, intranasally, intramuscularly and by spraying.

Statistical analysis

Results shows for birds vaccinated with Newcastle disease viral vaccine, the best route of vaccination is the ocular route (protection rate 82.35 %), followed by per os, intramuscularly, intranasally and at last spraying.

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دراسات سيرولوجية على تأثير طريقة تحصين لقاح فيروس النيوكاسل على الإستجابة المناعية في الدجاج

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يهدف هذا البحث إلى دراسة تأثير طرق الاستخدام المختلفة للقاح فيروس مرض النيوكاسل على القوة المناعية للطيور باستخدام الطرق السيرولوجية المختلفة وتوصي هذه الدراسة إلى استخدام التقطير في العين حيث أعطى أعلى معدل استجابة مناعية ضد فيروس مرض النيوكاسل – التحصين بالفم – الحقن العضلي ثم بالرش على الترتيب حيث أعطت نسبة صد في حالة اختبار التحدي بالعترة الضارية لفيروس النيوكاسل المعدي وكانت ٨٢,٣٥% ، ٧٢,٢% ، ٦٦,٧% ، ٦٣,١٦% ، ٥٦,٢٢% ، على الترتيب لطرق التحصين المختلفة.