BENHA VETERINARY MEDICAL JOURNAL, VOL. 34, NO. 2: 278-290, JUNE 2018

Evaluation of Locally Prepared Inactivated Newcastle Disease Virus Vaccine

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A B S T R A C T

Vaccination is one of the control measures of the disturbing Newcastle Disease in Egypt. Inactivated Newcastle Disease virus (NDV) vaccine was prepared using a local isolate (NDV-ch-EG-CLEVB-F604-2016), from Qaliobia governorate, Egypt, with Montanide ISA70VG as an oil adjuvant. The prepared vaccine was evaluated in comparison with an inactivated imported vaccine. Chick and Turkey chick groups vaccinated with either prepared or imported vaccines showed high serum antibody titers from the 3rd week post vaccination and reached the highest titer at the 9th week post vaccination using HI test. Both prepared and imported vaccines gave near percentage of protection against the local and the classical strain in both groups of chicks and turkeys ,21 days post vaccination, with no clinical signs or lesions on examination. Concluding that the locally prepared inactivated NDV vaccine can protect chicken and turkey against both homologous and heterologous challenging viruses.

Keywords: ND, evaluation, inactivated vaccine, Montanide ISA70VG.

(http://www.bvmj.bu.edu.eg) (BVMJ-34(2): 278-290, 2018)

1. INTRODUCTION

Newcastle disease virus (NDV) has a wide host species variety, including about 241 species of 27 orders, out of known 50 orders of birds *(Madadger et al., 2013)*. Most commonly affected species include chickens, turkeys, ducks, pigeons *(Zhang et al., 2011)*. NDV is a member of Avula virus genus, family Paramyxoviridae. Paramyxoviruses are single-stranded RNA, with a genome size of about 15 kb with a genomic arrangement of six genes coding for six polypeptides *(Lamb and Parks, 2002)*. The epizootic nature of ND disease has caused huge economic losses in poultry industry around the world since year 1920*(Mayo, 2002)*. The ND outbreaks are occurring frequently in Egypt and the source of the virulent NDV in these outbreaks is anonymous *(Mohamed et al., 2009)*.

The envelope of the Newcastle disease virus holds two surface glycoproteins:

Haemagglutinin-Neuraminidase (HN) which is responsible for attachment of the virus to the host cell receptors and fusion (F) protein which is responsible for fusion of viral envelope with the cellular plasma membrane. Both these two glycoproteins are the antigenic components against which neutralizing antibodies are directed *(Yusoff and Tan, 2001).* Vaccination, initially with inactivated virus, was considered a possibility for the control of ND at the time of the apparent emergence of the virus *(Placcidi and Sentucci, 1952*). Also, vaccination will protect birds from the more serious consequences of NDV infection since clinical signs are greatly diminished in relation to antibody level achieved *(Alexander,1997).*

This study was conducted for evaluation of locally prepared inactivated oil emulsion NDV vaccine from the newly isolated NDV strain (NDV-ch-EG-CLEVB-F604-2016).

1. Material and Methods:

1.1. Experimental Hosts:

1.1.1. Embryonated Chicken Eggs (SPF-ECE):

Specific Pathogen Free (SPF-ECE) ,9-10 day old, purchased from the SPF egg farm, Kom Oshim, EL-Fayoum, Egypt. The eggs were used for propagation and titration of ND viruses and ensuring of completion of virus inactivation of the tested inactivated ND vaccine.

1.1.2. SPF Chicks:

Total number of 150, one-day-old SPF chicks were purchased from SPF poultry farm, Kom Oshim, EL-Fayoum, Egypt. The chicks were maintained at CLEVB in positive pressure isolators with continuous light for evaluation of the tested ND vaccines.

1.1.3. *Turkey Chicks*:

A total number of 150, one day old turkey chicks, obtained from experimental and research farm, faculty of agriculture, Cairo university. They were kept under strict hygienic measures, provided with water and balanced ration. They were used for evaluation of prepared and commercial NDV vaccines.

1.2. Commercial Inactivated Newcastle Disease (ND) Vaccine:

It is an oil emulsion vaccine contains inactivated ND virus (CLONE 30 strain). The vaccine was produced by Intervet. It was administered S/C at the lower third of the neck in a dose of 0.5 ml/bird.

1.3. Antigens and Antisera:

A. ND antigens for Sheble and Reda virus (NDV-ch-EG-SR-76) and the Newly isolated NDV (NDV-ch-EG-CLEVB-F604-2016), were prepared *(OIE, 2017)* and their titers were 2^8 and 2^7 HA respectively. They were used in HI test.

B. Standard ND antisera were obtained from GD, Holland and used as positive control for evaluation of tested ND vaccines.

1.4. Challenge viruses:

A. Velogenic Viscerotropic NDV (VVNDV):

It is a local virulent ND strain (SR/76) isolated by (Sheble and Reda, *1976)* and was obtained from veterinary Serum & Vaccine Research institute, Abbasia, Cairo. Its titer was 10^9 EID_{50}/ml. The challenge dose was adjusted to be 10^6 EID_{50}/ml per bird and injected intramascular.

B. Locally isolated NDV (NDV-ch-EG-CLEVB-F604-2016) (ND-F604):

It was locally isolated at CLEVB in 2016 from Qaliobia Governerate and identified genetically by NLQP as virulent strain related to the groups of ND strains that appeared since 2012 under genotype VIId

reference strains. Its titer was $10^{9.7}$ EID_{50}/ml. The challenge dose was adjusted to be 10^6 EID_{50}/0.5ml per bird and injected intramuscular. It was identified under the Name of (NDV-ch-EG-CLEVB-F604-2016) with Accession Number MHO78055.

2. Preparation of an experimental batch of inactivated NDV vaccine:

A. Propagation of NDV in SPF-ECE (OIE,2017):

An inactivated oil emulsion ND vaccine was prepared using the local isolate (NDV-F604).

The virus was diluted 10fold dilution in sterile physiological saline pH 7.2, (0.1ml) of the virus suspension dilution was inoculated in to the allantoic sac of each of 10 days old SPF-ECE and incubated at 37°Cwith daily candling. The allantoic fluid of the inoculated eggs was harvested after 72 hrs. and examined for HA activity.

The harvested allantoic fluid was titrated in ECE and tested for sterility against any bacterial, fungal and mycolasmal contamination. The titer of the virus was adjusted to be 10^6 EID_{50}/dose for vaccine preparation.

B. Inactivation of the propagated NDV:

The harvested infected allantoic fluid was treated with binary ethylene amine (BEI - Sigma) at a final concentration of 0.002 mol/L, with continuous stirring during inactivation processacc.to (Bahnemann ,1990). Samples from the inactivated virus should be tested for completion of inactivation by passage (at least 2 blind passages) in to 10 days old, ECE (0.1 ml/egg) via the allantoic cavity route and examined for 5 days.

All the inoculated ECE were tested after incubation period for the presence of HA activity by rapid HA test after every passage. The virus is considered completely inactivated if there is no embryo mortality or HA activity.

C. Preparation of the vaccine emulsion:

It was prepared as water in oil emulsion(W/O) using Montanide™ ISA70 VG (SEPPIC, Pharmacy division, France batch No. 948400) at a ratio of3/7 (v/v) according to the standard protocol of SEPPIC for manufacture instruction.

3. Evaluation of the prepared and commercial inactivated NDV oil emulsion vaccines:

Testing the quality control of the prepared and commercial inactivated NDV vaccine including sterility and safety which was carried out according to (OIE, 2017) and Egyptian standard regulation for veterinary Biologics (2009).

A. Sterility test:

It was applied to confirm that the prepared and the commercial ND inactivated vaccines were free from bacterial and fungal contamination. Samples from the tested vaccines inoculated into nutrient agar and thioglycolate broth media then incubated at 37°C for detection of any bacterial contamination. Other samples were cultured on Saburaoud agar media and incubated at 25°C for detection of any fungal contamination. The inoculated media were inspected daily for any possible growth.

B. Safety test in chicks:

Groups of 3 weeks old chicks were inoculated S/C with double the field dose of the tested vaccines. Another group of chicken were kept unvaccinated as control. All the chicks were observed for 21 days

for any signs of local reaction or appearance of any clinical signs of NDV.

C. Potency of the prepared vaccine:
Groups of SPF chickens and turkeys (3 wks old) were vaccinated S/C with the field dose recommended by the producer of the tested ND vaccines. Blood samples were taken weekly for serological analysis of ND Ab level using HI test. Three weeks post vaccination, the vaccinated and the control chicken and turkeys were challenged with 10^6 EID_{50} /0.5ml of both the Classical VVNDV (SR/76) and the newly isolated (NDV-F604) viruses intramuscular. All the dead and the clinically infected birds were recorded during the observation period (2wks) for detection of the protection %.

4. Experimental Design:
In this study (110) SPF chicken and (110) turkeys were used to evaluate the efficacy of locally prepared and commercial inactivated ND vaccines. The vaccinated chicken and turkey groups were divided to 2 groups.

The first group (40 bird) was vaccinated with locally prepared inactivated ND vaccine and the second group (40 birds) was vaccinated with the commercial inactivated ND vaccine. While the control group (30 birds) either of chicken or turkey. All the 3 groups were subdivided in to 3 subgroups. The 1^{st}&2^{nd} subgroups (15 bird/each) were challenged with the Classical local VVNDV & newly isolated (NDV-F604) virus respectively, the 3^{rd} subgroup kept for serological analysis. The control groups (of each chicken and turkeys) were subdivided into 3 subgroups (10/each), the 1^{st}&2^{nd} subgroups were infected with the same previously mentioned challenge viruses and the 3^{rd} subgroup was kept for control negative serum as shown in (Table 1).

3. RESULTS

3.1 Sterility test:
By examination of the cultured media with the tested inactivated ND vaccines, it didn't show presence of any bacterial & fungal contamination.

3.2 Safety test:
The safety examination of the tested ND vaccines demonstrated that the injected chicks and turkeys didn't show any local or adverse systemic reactions due to any viral diseases during the observation period.

3.3 Results of potency test:
The mean HI antibody titers of the tested inactivated ND vaccines used for vaccination of chicken were explained in (Tables 2,3).

It was observed from (Table 2) that in case of locally prepared ND inactivated vaccine, the mean HI antibody titer increased from 0 at pre-vaccination time to (6 log2) at 3 WPV and was still increasing till 9^{th} WPV (10 log2) when using (NDV-F604) Ag. While the mean Ab titer of chicken vaccinated with inactivated commercial ND vaccine increased gradually from (3 log2) at 1^{st} WPV to reach (10.1 log2) at 9^{th} WPV when using the same Ag.

Also, it was found that the mean Ab titer for inactivated ND vaccines detected by Classical VVNDV (SR/76) ND Ag were shown in Table (3). It

was observed that the Ab titers were increased gradually from $2^{2.5}$& 2^{3} at 1st WPV to become (2^{10}) & ($2^{10.4}$) at 9th WPV for inactivated locally prepared & commercial ND vaccines, respectively.

On the other side the immune response against the tested ND vaccines measured in turkey was cleared in (Tables 4,5). Among the group of turkey vaccinated with ND vaccines, it was found that the mean HI antibody titer was increased to reach the maximum at 9th WPV ($2^{10.2}$) for both inactivated locally prepared & commercial ND vaccines when examined by the local (ND-F604) Ag, (Table4).

3.4 Results of ND Vaccines Efficacy:

Results of challenge test of chicken groups vaccinated with local & commercial ND vaccines using the isolated (ND-F604) virus described in (Table 6). It was evident that by challenging the immunity of chicken groups vaccinated with both the locally prepared & the commercial ND vaccines, (100% & 93.3%) of the chicken of each group respectively were protected against the disease for 10 days post challenge in comparison to the control group (0% protection).

Meanwhile, the mean HI Ab. Titers measured by Classical (SR/76) ND Ag in sera of the vaccinated & control turkey were reaching the maximum level (10.2 for local vaccine & 10.5 for commercial vaccine) as shown in (Table 5).

While the protection % of the same chicken groups were (93.3%) for each, after challenging with the locally isolated with VVNDV (SR/76) virus (Table7).

Meanwhile the results of challenge test for evaluating the efficacy of inactivated locally prepared & commercial ND vaccines in turkey were described in Table (8,9). It was noticed that the protection % of the vaccinated turkeys with the locally prepared and the commercial ND vaccines was (93.3%) for both after challenge either with (ND-F604) virus or the Classical VVNDV (SR/76) (Table 9).

Table (1): Experimental design of the study:

Host group	Group ID	Group No.	Subgroup ID	Subgroup No.	Treatment
Chicken	A	40	1	15	Vaccinated with prepared NDV vaccine & challenged with VVNDV
			2	15	Vaccinated with prepared NDV vaccine & challenged with F604-M2-NDV
			3	10	Vaccinated with prepared NDV vaccine & unchallenged.
	B	40	4	15	Vaccinated with commercial NDV vaccine & challenged with VVNDV
			5	15	Vaccinated with commercial NDV vaccine & challenged with F604-M2-NDV
			6	10	Vaccinated with prepared NDV vaccine & unchallenged.
	Control	30	7	15	Unvaccinated & infected with VVNDV
			8	15	Unvaccinated & infected with F604-M2-NDV
			9	10	Unvaccinated & Unchallenged.
Turkey	C	40	1	15	Vaccinated with prepared NDV vaccine & challenged with VVNDV
			2	15	Vaccinated with prepared NDV vaccine& challenged with F604-M2-NDV
			3	10	Vaccinated with prepared NDV vaccine & unchallenged.
	D	40	4	15	Vaccinated with commercial NDV vaccine & challenged with VVNDV
			5	15	Vaccinated with commercial NDV vaccine & challenged with F604-M2-NDV
			6	10	Vaccinated with prepared NDV vaccine & unchallenged.
	Control	30	7	15	Unvaccinated & infected with VVNDV
			8	15	Unvaccinated & infected with F604-M2-NDV
			9	10	Unvaccinated & Unchallenged.

Table (2): Results of HI test of vaccinated chicken group with the inactivated locally prepared NDV Vaccine using (ND-F604) Ag:

Vaccine	No. of chicken	Mean HI titre / WPV								
		1	2	3	4	5	6	7	8	9
Locally prepared vaccine	10	$2^{2.5}$	2^{4}	2^{6}	$2^{7.5}$	$2^{8.2}$	$2^{8.5}$	2^{9}	$2^{9.5}$	2^{10}
Imported vaccine	10	2^{3}	$2^{4.6}$	$2^{6.1}$	$2^{7.7}$	$2^{8.3}$	$2^{8.8}$	$2^{9.2}$	$2^{9.5}$	$2^{10.1}$

Control	10	0	0	0	0	0	0	0	0	0

Table (3): Results of HI test of vaccinated chicken group with the inactivated locally prepared NDV Vaccine using (SR/76) ND Ag:

Vaccine	No. of chicken	Mean HI titre / WPV								
		1	2	3	4	5	6	7	8	9
Locally prepared vaccine	10	$2^{2.5}$	$2^{4.2}$	$2^{6.3}$	$2^{7.8}$	$2^{8.5}$	$2^{8.7}$	$2^{9.2}$	$2^{9.5}$	2^{10}
Imported vaccine	10	2^{3}	$2^{4.8}$	$2^{6.4}$	$2^{7.9}$	$2^{8.6}$	$2^{8.8}$	$2^{9.4}$	$2^{9.7}$	$2^{10.4}$
Control	10	0	0	0	0	0	0	0	0	0

Table (4): Results of HI test of vaccinated Turkey group with the inactivated locally prepared NDV Vaccine using (ND-F604) Ag:

Vaccine	No. of Turkey	Mean HI titre / WPV								
		1	2	3	4	5	6	7	8	9
Locally prepared vaccine	10	2^{3}	$2^{4.5}$	$2^{6.2}$	$2^{7.7}$	$2^{8.2}$	$2^{8.8}$	$2^{9.2}$	$2^{9.7}$	$2^{10.2}$
Imported vaccine	10	$2^{3.5}$	2^{5}	$2^{6.3}$	$2^{7.8}$	$2^{8.3}$	$2^{8.9}$	$2^{9.5}$	$2^{9.7}$	$2^{10.2}$
Control	10	0	0	0	0	0	0	0	0	0

Table (5)**:** Results of HI test of vaccinated Turkey group with the inactivated locally prepared NDV Vaccine using (SR/76) ND Ag:

Vaccine	No. of Turkey	Mean HI titre / WPV								
		1	2	3	4	5	6	7	8	9
Locally prepared vaccine	10	$2^{2.7}$	$2^{4.6}$	$2^{6,5}$	2^{7}	2^{8}	$2^{8.4}$	$2^{9.1}$	$2^{9.7}$	$2^{10.2}$
Imported vaccine	10	$2^{3.3}$	$2^{4.8}$	$2^{6.8}$	$2^{7.5}$	$2^{8.3}$	$2^{8.8}$	$2^{9.4}$	$2^{9.9}$	$2^{10.5}$
Control	10	0	0	0	0	0	0	0	0	0

Table (6): Results of challenge test of vaccinated chicken and challenged with the (ND-F604) virus:

Vaccine type	No. of birds	Daily observation of chicken group										Total no. of dead birds	Protection %
		1	2	3	4	5	6	7	8	9	10		
Locally prepared vaccine	15											0	100 %
Imported vaccine	15								1			1	93.3%
Control	10				6	4						10	0%

Table (7): Results of challenge test of vaccinated chicken and challenged with Classical VVNDV (SR/76) virus:

Vaccine type	No. of birds	Daily observation of chicken group										Total no. of dead birds	Protection %
		1	2	3	4	5	6	7	8	9	10		
Locally prepared vaccine	15					1						0	93.3 %
Imported vaccine	15						1					1	93.3%
Control	10				8	2						10	0%

Table (8): Results of challenge test of vaccinated turkey and challenged with locally isolated NDV (ND-F604):

Vaccine type	No. of birds	Daily observation of turkey group										Total no. of dead birds	Protection %
		1	2	3	4	5	6	7	8	9	10		
Locally prepared vaccine	15							1				1	93.3 %
Imported vaccine	15									1		1	93.3%

Control	10			5	5						10	0%

Table (9): Results of challenge test of vaccinated turkey and challenged with local standard VVND (SR/76) virus:

Vaccine type	No. of birds	Daily observation of turkey group										Total no. of dead birds	Protection %
		1	2	3	4	5	6	7	8	9	10		
Locally prepared vaccine	15				1							1	93.3 %
Imported vaccine	15									1		1	93.3%
Control	10				7	3						10	0%

4.DISCUSSION

ND is considered one of the most important poultry viral respiratory disease causing severe lesions and high mortality in-between the bird flocks. In the last several yours, ND has caused great worldwide lesion in poultry *(Brown and Bevins, 2017).*

In Egypt, NDV outbreaks are occurring frequently and the epidemiology of the virulent NDV Isolates from these out breaks was elucidated. *(Radwan et al., 2013).* Vaccination was used in Egypt as a common tool to prevent or reduce losses due to ND infection *(Abd El Aziz et al., 2016).*

Also, vaccination strategy has an important role in the limitation of viral shedding and subsequently, minimize the spread of infection to the surrounding environment *(Miller et al.,2010).* A variety of vaccines are used to control the disease in chicken and turkey as live attenuated and inactivated ND vaccines to control the outbreaks caused by virulent ND viruses *(Allan et al., 1973).*

In this study, an inactivated ND vaccine was prepared using the locally isolated ND virus at 2016(ND-F604), then its efficacy was compared with that of the imported commercial vaccine for protection of either chicken or turkey against the ND infection.

All the tested inactivated ND vaccines approved that they were safe, sterile, pure and valid for use *(OIE, 2017).*

Both F and HN glycoproteins are the antigenic components against which neutralizing antibodies are directed *(Yusoff and Tan, 2001)*

In the current study, the ability of locally prepared and commercial inactivated ND vaccines in induction of good protective immune response for chicken and turkey were tested. That immune response of both ND vaccines was determined passing on the serology performed weekly after vaccination using both ND Ags (ND-F604 and SR/76) as shown in (Table 2, 3, 4, 5).

The HI test considered the most convenient serological method for detection of the immune response against AIV and NDV vaccines *(Tang et al., 2005).*

It was found that from (Table 2, 3) that the mean HI Ab titers of chicken vaccinated with local ND vaccine were (10^{10}) at 9^{th} WPV when tested either by the local and stand local Ags, also in case of commercial ND vaccine the Ab titers were ($10^{10.1}$ to $10^{10.4}$) at 9^{th} WPV against both local and standard Ags (ND-F604 and SR/76) respectively.

The same results were observed in vaccinated turkey with the local and commercial ND vaccines (Table 4, 5), the Ab titers were ($10^{10.2}$) at 9^{th} WPV in case of local ND vaccine when tested by both local and stand Ags. While the Ab titers were ($10^{10.2}$ and $10^{10.5}$) at 9^{th} WPV in case of commercial ND vaccine when examined by local and standard Ags (ND-F604 and SR/76) respectively.

From the present work it was observed that the locally prepared and the commercial ND vaccines produce nearly the same Ab titers when examined by the local and standard Ags (ND-F604 and SR/76) in chicken or turkey. These observations are supported by previous study which showed that the antigenic similarity is shared among all NDV strains and isolates will cross-protect against other NDV isolate *(Courtney et al., 2012).*

The efficacy of the inactivated local and commercial ND vaccines examined by challenge tests was cleared in (Tables 6, 7, 8, 9). The protection % of local ND vaccine was 100% and 93.3% against the local and the standard challenge viruses in chicken host and in turkey host was 93.3% for both. Meanwhile, all chicken and turkey immunized by commercial ND vaccine as one dose were protected by 93.3% against both local and standard challenge viruses.

The previous results demonstrated that the inactivated ND vaccines (either locally prepared or commercial) induced a sufficient effective protection for chicken and turkey against both local and standard challenge viruses and this agreed with *(Hu et al.,2011)* who mentioned that the heterologous vaccines

can prevent infection and viral transmission if sufficient time is allowed for bird to mount a proper immune response beside the use of homologous Ags.

Furthermore, it must focus on ways to accelerate speed of the immune response evoked beside the use of homologous Ags. Also, when flock immunity increases, even low level of Ab titers may be sufficient to prevent infection depending on the challenge dose (Miller et al., 2010). Another important point was found by (Miller et al., 2013) is that Lasota vaccines induce the lowest pre-challenge Ab levels, however there was in most cases 100% protection against mortality and clinical signs but not effective in protecting against viral replication and transmission.

Finally, virulent NDV continues to be endemic in Egypt and many countries around the world despite the application of billions of doses of live and inactivated ND vaccines. Also, it is necessary to note that the increase in variability in the HN protein compared with the F protein can affect the cross protection. So, the selection of vaccinal Ags must be done on the basis of the cross-protection studies with live animals.

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