Studies on the Efficacy of a recombinant Marek's disease- Newcastle disease vaccine in Broiler chickens

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Abstract

This study was planned to evaluate the application of the recombinant Marek's disease-Newcastle disease (rMD-ND) vaccine in broiler chicken either alone or as a prime boost for other ND vaccines in vaccination programs against ND. Six batches of rMD-ND (A, B, C, D, E and F) were selected from CLEVB with variable efficacy levels in SPF chickens all over 3 years (2 batches per year). One day old (DO) broiler chicks were vaccinated with different vaccination programs against ND. The chicken groups were challenged at 28th day old with a velogenic viscerotropic NDV. The protection rates were 90%, 95% and 90% for batches coded B, C and E when applied alone and improved the protection has improved when used as prime boost for vaccination program. On the other hand the protection offered by batches A, D and F of the r. MD-ND vaccine alone were 40%, 50% and 60% respectively and these batches did not provide any improvement for the other ND vaccines when used in programs wither other conventional vaccines.

Key words: efficacy; recombinant Marek's disease- ND vaccine; Broiler chickens.

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Introduction

Nowadays, poultry industry is constantly dealing with a plethora of avian pathogens with the potential impacts on the economic viability and productivity of this industry. Mortality and condemnation losses resulting from field outbreaks of Marek's disease and Newcastle are given greatest priority among a multitude of problems which threaten this industry worldwide (Xiao et al., 2014).

Newcastle Disease (ND) is contagious disease caused by Newcastle Disease Virus (NDV) belonging to genus Avulavirus in the family paramyxoviridae, result in high mortalities which may reach to 100% (Mayo, 2002 and Alexander, 2011).

The present study aims in assessment of efficacy of rMD-ND vaccine, could it add value considering the improvement of vaccination strategy (alone or with other NDV vaccines- live NDV vaccines [LaSota and Hitchner] and or inactivated one) against ND in broiler chickens?

Material and Method

Ethical approval

Institutional Animal Ethics committee has accorded permission for conducting this trial.

Bio-risk approval

Bio-risk committee in CLEVB has accorded permission for conducting this trial.

1. Vaccines

1.1. Recombinant Marek-Newcastle disease (rMD-ND) vaccines

Six commercial recombinant vaccines of 6 different batches received by CLEVB for routine evaluation before release to market use.

1.2. Live ND Vaccines

Six ND vaccines were used in this study including; three commercial live LaSota and three commercial live Hitchner vaccine. These vaccines should satisfactory result by CLEVB.

1.3. Inactivated vaccines

Three batches of inactivated NDV vaccines were used.

2. Virulent Newcastle Disease virus

Velogenic viscerotropic ND virus (VVNDV), SR/76 strain (Hussein et al., 2005) was used for challenge test. The challenge dose was 0.5 ml containing 10^4 EID_{50}/bird via intramuscular inoculation. The virus obtained from Strain Bank Department, CLEVB. It was titrated in specific pathogen free embryonated chicken eggs (SPF-ECE) as described by (Villegas, 1990), the infectivity titer was as calculated according to the method of (Reed and Muench 1938).

3. Newcastle disease heamagglutinating antigen

NDV antigen, LaSota strain was obtained from Strain Bank Department, (CLEVB). The antigen was used with a titer of 4 HAU according to (OIE 2012).

4. Serological test

4.1. Haemagglutination test (HA)

It was carried out according to the standard procedure described by *(Anon, 1971).*

4.2. Haemagglutination inhibition test (HI)

It was carried out for evaluation of the seroconversion against the NDV vaccines according to (OIE 2012).

5. Experimental host

One day old commercial Hubbard broiler chicks were obtained from Cairo Poultry Company (CPC). They were reared in cages under hygienic condition in previously cleaned and disinfected isolated experimental rooms. Water and feed were provided adlibitum. The challenge was conducted in SPF isolator in specific CLEVB animal care building with Bio risk guideline.

6. Tissues cultures and cell culture media

Primary chicken embryo fibroblast cell (CEF) was obtained from Central Lab for Evaluation of Veterinary Biologics (CLEVB); which was prepared as described by (Schat and Purchase, 1989). Tissue culture was used for titration of Marek's disease virus in the vaccine using microtiter technique according to (Villegas, 1990).

7. Experimental design

For each year two batches of the recombinant MD-ND vaccine with different efficacy will be evaluated through application of different vaccination programs using ND vaccines of same production year in 360 broiler one day old (DO) chicks that divided into (12) groups each of 30 birds (per year 2013, 2014 and 2015) as shown in tables (1, 2 and 3).

Table (1): First experimental vaccination against ND with different vaccination programs using batches of 2013

Group No.	Recombinant vaccine batch (0 DO)	Hitchner vaccine (7 DO)	Inactivated ND vaccine (7 DO)	LaSota vaccine (18 DO)
G1	A+	-	-	-
G2	A+	-	-	+
G3	A+	+	-	+
G4	A+	-	+	-
G5	B+	-	-	-
G6	B+	-	-	+
G7	B+	+	-	+
G8	B+	-	+	-
G9	-	-	-	+
G10	-	+	-	+
G11	-	-	+	-
G12	-	-	-	-

Table (2) Second experimental vaccination against ND with different vaccination Programs using batches of 2014

Group No.	Recombinant vaccine batch (0 DO)	Hitchner vaccine (7 DO)	Inactivated ND vaccine (7 DO)	LaSota vaccine (18 DO)
G1	C+	-	-	-
G2	C+	-	-	+
G3	C+	+	-	+
G4	C+	-	+	-
G5	D+	-	-	-
G6	D+	-	-	+
G7	D+	+	-	+
G8	D+	-	+	-
G9	-	-	-	+
G10	-	+	-	+
G11	-	-	+	-
G12	-	-	-	-

Table (3) Third experimental vaccination against ND with different vaccination Programs using batches of 2015

Group No.	Recombinant vaccine batch (0 DO)	Hitchner vaccine (7 DO)	Inactivated ND vaccine (7 DO)	LaSota vaccine (18 DO)
G1	E+	-	-	-
G2	E+	-	-	+
G3	E+	+	-	+
G4	E+	-	+	-
G5	F+	-	-	-
G6	F+	-	-	+
G7	F+	+	-	+
G8	F+	-	+	-
G9	-	-	-	+
G10	-	+	-	+
G11	-	-	+	-
G12	-	-	-	-

- Blood samples were collected at one (DO) and just before challenge from all groups and sera were kept at -20 C till examined.
- The challenge test was carried out at the 28th day of age for all groups using 20 birds group.
- The challenged groups were observed for 14 days post challenge, where clinical signs and mortalities were recorded.

Results

HI test revealed that maternal antibody titers were 8, 7.8 and 8 $\log_2$ at the first day of age in broiler chicks of the first, second and 3rd experiments respectively. Considering the potency of the six rMD-ND vaccines, the titers of the MDV of the vaccines were 2000, 3000, 3100, 2100, 3000 and 2200 PFU/dose for batches A, B, C, D, E and F, respectively.

In the first experiment the batch A of the rMD-ND Vaccine was proved to be unsatisfactory as inducing 40% protection against NDV when used alone, Also no improvement was recorded in the efficacy of the other vaccination programs against ND when the same recombinant vaccine batch was added to other conventional in vaccination programs.

When Batch B of the rMD-ND vaccine was used alone resulted in 90% protection and HI titer for other vaccination programs against ND when that batch was added to them as illustrated in Table (4) and chart (1).

Nearly the same results were recorded in the second and third experiments when rMD-ND vaccines induced protection percentages 50% and 60% against NDV respectively (Tables 5 and 6).

Table (4): Protection percentages and ND HI antibody titers (Log_2) in broiler chickens vaccinated with rMD-NDV vaccines with different vaccination programs against ND (first Experiment)

Group No.	No. of challenged birds	Recombinant vaccine batch (0 DO)	Hitchner vaccine (7 DO)	Inactivated ND vaccine (7 DO)	LaSota vaccine (18 DO)	HI Titre (log_2)	Protection %
G1	20	A+	-	-	-	1.5	40
G2	20	A+	-	-	+	4.5	65
G3	20	A+	+	-	+	6.3	90
G4	20	A+	-	+	-	5	80
G5	20	B+	-	-	-	5	90
G6	20	B+	-	-	+	7.5	95
G7	20	B+	+	-	+	8.5	100
G8	20	B+	-	+	-	7	95
G9	20	-	-	-	+	4.3	65
G10	20	-	+	-	+	6.1	90
G11	20	-	-	+	-	5.3	80
G12	20*	-	-	-	-	0	0
	10**	-	-	-	-	-	-

* Control positive

** Control negative

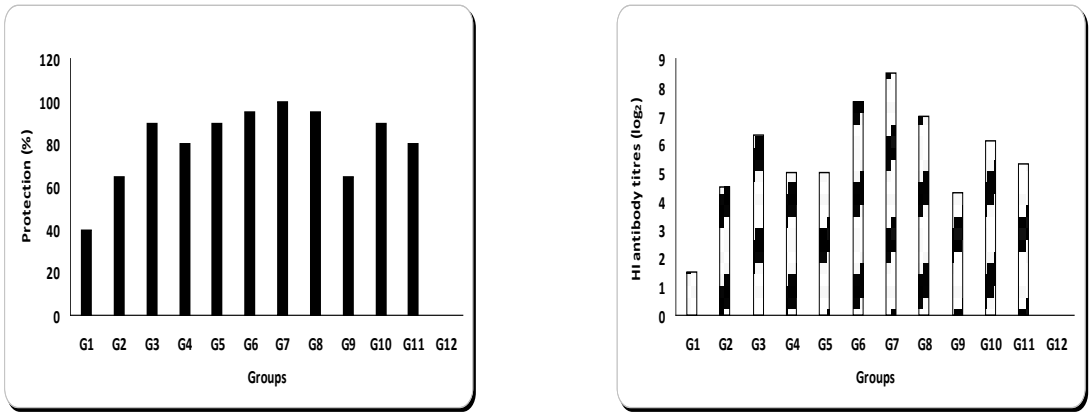


Chart (1): Protection percentages and ND HI antibody titers (Log2) in broiler chickens vaccinated with rMD-NDV vaccines with different vaccination programs against ND (first Experiment- Batches of 2013).

Table (5): Protection percentages and ND HI antibody titers (Log_2) in broiler chickens vaccinated with rMD-NDV vaccines with different vaccination programs against ND (second Experiment)

Group No.	No. of challenged birds	Recombinant vaccine batch (0 DO)	Hitchner vaccine (7 DO)	Inactivated ND vaccine (7 DO)	LaSota vaccine (18 DO)	HI Titre (log_2)	Protection %
G1	20	C+	-	-	-	5	95
G2	20	C+	-	-	+	6.5	100
G3	20	C+	+	-	+	8	100
G4	20	C+	-	+	-	8	95
G5	20	D+	-	-	-	1.9	50
G6	20	D+	-	-	+	4.3	65
G7	20	D+	+	-	+	6.2	90
G8	20	D+	-	+	-	5.3	80
G9	20	-	-	-	+	4.3	65
G10	20	-	+	-	+	6.1	90
G11	20	-	-	+	-	5.3	80
G12	20*	-	-	-	-	0	0
	10**	-	-	-	-	-	-

* Control positive
** Control negative

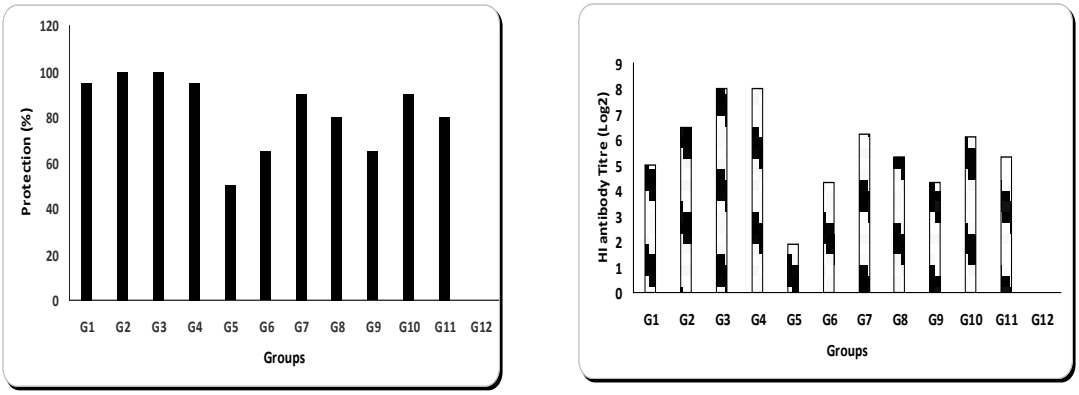


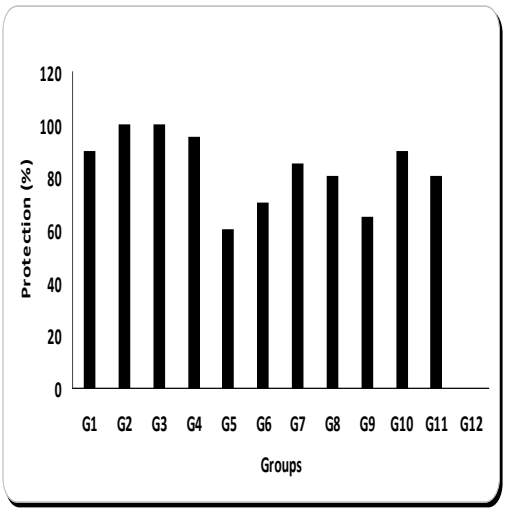
Chart (2): Protection percentages and ND HI antibody titers (Log_2) in broiler chickens vaccinated with rMD-NDV vaccines with different vaccination programs against ND (second Experiment- Batches of 2014).

Table (6): Protection percentages and ND HI antibody titers (Log_2) in broiler chickens vaccinated with rMD-NDV vaccines with different vaccination programs against ND (Third Experiment)

Group No.	No. of challenged birds	Recombinant vaccine batch (0 DO)	Hitchner vaccine (7 DO)	Inactivated ND vaccine (7 DO)	LaSota vaccine (18 DO)	HI Titre (log_2)	Protection %
G1	20	E+	-	-	-	5.5	90
G2	20	E+	-	-	+	7.5	100
G3	20	E+	+	-	+	8.3	100
G4	20	E+	-	+	-	7.8	95
G5	20	F+	-	-	-	2	60
G6	20	F+	-	-	+	5	70
G7	20	F+	+	-	+	5.5	85
G8	20	F+	-	+	-	5.4	80
G9	20	-	-	-	+	4.3	65
G10	20	-	+	-	+	6.1	90
G11	20	-	-	+	-	5.3	80
G12	20*	-	-	-	-	0	0
	10**	-	-	-	-	-	-

* Control positive

** Control negative



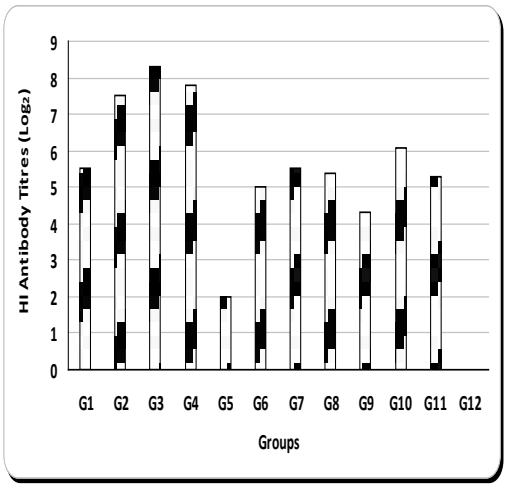


Chart (3) Protection percentages and ND HI antibody titers (Log2) in broiler chickens vaccinated with Rec.MD-NDV vaccines with different vaccination programs against ND (Third Experiment - Batches of 2015).

Discussion

Allover many decades ND still endemic in Egypt, in spite of application of numerous vaccination programs in boiler, layer and breeder chicken flocks. Each vaccination program is designed to use more than one vaccination shoot and more than one type of ND vaccines either live or inactivated or both.

The results of the present study clarifies that batches of the rMD-NDV vaccine (B, C and E) that induced acceptable protection percentages in the broiler chickens and improved the protection and HI antibody titers when used with other conventional vaccines. These results are similar to that obtained in SPF chickens by CLEVB, indicating that the maternally derived (MD) antibodies at DO of the commercial chicks did not interfere with rMD-ND vaccine, although vaccination was carried out at one day old (Morgan et al. 1992).

On the other hand, when the other 3 batches of r MD-ND vaccines with low efficacy levels in SPF according to CLEVB results showed 40%, 50% and 60% protection when used alone. The reported results disagreed with that of (Sonoda et al., 2000) who found that the use of rMDV-F vaccine induce high protection against challenge with velogenic NDV. The lower protection induced by the tested batches could be due to the lower titer of the vector (MDV) which were 2000, 2100 and 2200 PFU/dose; respectively compared with these of the batches B, C and E of higher titers not less than 3000 PFU/dose. These results highlight the importance of the vector titer of rMD-NDV vaccines.

No improvement in the protection % and HI titers were recorded when these batches used as a prime boost for different vaccination programs against ND, as illustrated in tables 4,5 and 6 and charts 1,2, and 3. These results confirm that the use vaccines with low quality will induce poor protection in the vaccinated chickens and might cause negative impact on poultry industry.

Conclusion

All the experiments were done under controlled laboratory conditions and the obtained results may comply with or differ from that under field conditions, however according to the obtained results it can be conclude that: The rMD-ND vaccine batches that proved to be effective in SPF chickens were effective also in broilers with MDA and improved the efficacy of the other vaccination programs against ND and the reverse was true for the unsatisfactory batches. The vector titer should be considered in the evaluation process and a cut off value must be addressed.

Author contributions

All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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