Vet.Med.J.,Giza. Vol.41,No.1.(1993):5-12.

STUDIES ON THE DEVELOPMENT OF NEWCASTLE DISEASE KILLED VACCINE

BY

MOHAB, AWAD*; A.M.SAMI**; AHMED EL-SANOUSI**; N.M. BARHOMA*; SALWA EL-ASSILY*; ENSAF M. KHASHABAH*; FEKRIA EL-BORDINY*; NADIA M. HASSAN*; AFAF H.A. ABDEL HADY* AND I.M. REDA**

* Vet. Serum & Vaccine Research Inst., Abbassia, Cairo.

** Faculty of Vet. Med., Cairo University

(Received: 9.10.1992).

SUMMARY

The present investigation deals with these items:

1. Effect of Beta propiolactone (BPL) as inactivating material on different strains of Newcastle Disease virus NDV (Velogenic, mesogenic, lentogenic, La-Sota and B_1).

2. Preparing oil adjuvant inactivated vaccine inactivated with BPL using La Sota strain of NDV. The end concentration of BPL vaccine was 0.2%

3. The prepared vaccine proved to be sterile and safe as controlled respectively by bacteriological test and inoculation into embryonated chicken eggs and 5 weeks old chickens.

4. The immune response HI titre of chickens inoculated S/C with BPL-inactivated oil adjuvant NSD vaccine at 7 weeks old, gradually increased for the first week, reached maximum at the fourth week after inoculation (GM 7.1) then gradually dropped to 6.4 at the 6th week post vaccination (P.V). Challenge test at 3 weeks and 6 weeks P.V. resulted in 100% protection in vaccinated chicks challenged 3 weeks P.V. and 90% protection in vaccinated chicks challenged 6 weeks P.V.

INTRODUCTION

Newcastle disease has been recognized as one of the most drasting disease of poultry industry. In Egypt the disease was reported by **Daubney and Mansi in (1947)** by the begnning of 1960 it become endemic **(Eissa, 1960, Sheble, 1960)**. Control policy has resorted to vaccination with live, locally manufactured F and Komarov and imported (B_1 and La Sota) vaccines **(Ahmed and Sabban, 1965; El-Saiad, 1984; Eissa, 1960; Sheble, 1960; Zaher et al., 1962).**

Beside the living vaccines, the introduction of inactive NDV vaccine in Egypt may be of paramount significance in controlling many diseases transmitted via utilization of non specific pathogen free eggs (SPF) for production of living vaccine **(Saleh, 1980)**. Many physical and chemical agents were used for the preparation of inactive NDV vaccines.

Therefore, the aim of the present work was oriented into the following:

1. Studying the effect of BPL in different concen-

trations on different strains of ND virus.

2. Preparation of potent inactive vaccine from La Sota strain by using oil adjuvant, the inactivated vaccine virus by Beta propiolactone.

3. Investigating the immunogenicity of prepared vaccine in immunocompetent chickens.

MATERIAL AND METHODS

1. Embryonated chicken eggs (ECE):

9-11 old ECE were obtained from General Poultry Company, used for titration of NDV strains and vaccine production.

2. Viruses: A) Vaccinal strains:

-La Sota strain imported from TAD and titrated in our laboratory was used throughout this investigation. The virus titre was $10^{9.5}$ EID50/ml. and preserved at 4°C until its use. The lentogenic living Hitchner B_1 strain was prepared locally in Vet. Serum and Vaccine Res. Inst., Abbassia, Cairo. The titre was $10^{10.5}$ EID_{50}/ml.

b) Virulent strain:

The locally isolated virulent velogenic viscerotropic (V.V) strain of Newcastle disease virus, was used as challenge virus in this study, isolated identified and typed by **"Sheble and Reda, 1978"**. Its titre in embryonated chicken eggs was 10^8 EID_{50}.

3. Beta propriolactone (BPL) was kindly obtained from the NAMRU-3, Abbassia, Cairo in a liquid form (2ml./vial) stored at -20°C, and used as viral inactivator at end concentration of 0.2%, 0.05% as well as 0.01% for vaccine production, it has been utilized at a concentration of 0.2%.

4. Tween 80: non ionic detergent was used as emulsifying agent to decrease the viscosity of the prepared vaccine.

5. Mineral oil "Risella 17": light mineral oil of low viscosity and lanolin as stabilizing agent suitable to water-in-oil emulsion resemble the Incomplete Fruend's prepared locally.

6. Phosphate buffer: Stock solutions **(Cruickshank et al., 1975).**

METHODS:

1. Propagation of the virus in Embryonated chicken eggs: The method which was reported by **Allan et al. (1973)** was used in this study.

2. Sterility test:

It was carried out according to **Allan et al. (1973).**

3. Infectivity titration in embryonated chicken eggs:

Estimation of 50% end point was carried out by the method of **Reed and Muench (1938).**

4. Inactivation of clarified virus suspension with BPL:

This was carried out according to **Schamidt and Lennette (1967).**

Inactivation of virus suspension with BPL:

0.8 ml. of stock BPL solution (10%) were mixed with 19.2 ml. of distilled water to reach a concentration of 0.4%. The 10 ml. of BPL solution (0.4%) were then added to 20 ml. of virus A.F. to achieve an end concentration of 0.2%. The pH was adjusted to 7.2. The mixture kept at 37°C under continuous stirring. After about 35 minutes, the fluid was transferred to another sterile beaker and stirring was continued for 3 hours at 37°C. The inactivated fluid was then kept at 4°C until tested for safety.

5. Mixing the inactivated fluid with Incomplete Fruend's adjuvant:

Equal parts of the oil adjuvant and the inactivated virus suspension were emulsified manually after addition of Tween 80 at an end concentration of 1% as emulsifying agent and to decrease the vis-

cosity of the vacine.

6. Safety test for vaccine preparation:

This was accomplished by inoculation of undiluted BPL-inactivated suspension into 25, nine-day-old embryonated chicken eggs (0.2 ml./egg) via the allantoic sac. 6 days post inoculation eggs were individually tested for HA activity by the rapid HA test. Fluids harvested from dead and survivor embryos were pooled and passaged blindly for 2 successive further passages before the batch of vaccine prepared was considered safe.

7. Haemagglutination test (HA):

It was carried out according to the Standard method described in (Method for Examination of Poultry Biologics and for Identifying and **Quantifying Avian Pathogens, 1971).**

8. Haemagglutination inhibition test (HIT):

It was carried out according to the standard method of Examining Poultry **Biologics,**

9. Virus-re-isolation:

Internal organs of dead challenged birds were used throughout this investigation for virus re-isolation. The allantoic route of inoculation was used for the isolation of NDV **(Fabricant, 1956).** On primary isolation, 3 blind passages in 9-11 days old chicken embryos were carried out. The specificity of embryonic death was determined by the detection of haemagglutinins in the allantoic fluid harvested from dead embryos. Embryos which survived the observation period were also subjected to the haemagglutination test.

10. Mean death time test (MDT):

It was carried out according to (Methods for Examining Poultry **Biologics, 1971).**

11. Intracloacal test in 6 weeks old chicks:

This test was carried out according to the method of **McDaniel and Osborn (1973).**

EXPERIMENTAL WORK AND RESULTS

Experiment I:

Determination of the inactivation time of different strains of Newcastle disease virus (NDV) with different concentration of BPL:

In this experiment the lethal effect of different concentration of B-propiolactone (0.2%, 0.05%, 0.01%) were tested on different strains of NDV with different pathogenicity levels (VVNDV, Komarov, La Sota and B_1 strains). The virus material in the form of infective amonioallantoic fluids are mixed with the determined concentration of BPL. The virus/BPL misture was incubated at 37°C under continuous stirring. Samples were collected on "O" time as well as at hourly intervals. The aliquots were used to determine quantitatively virus infectivity titre by inoculation into embryonated chicken eggs. Besides, he same samples were tested for the virus HA activity by the quantitative HA test. The duration of the experiment extended from 3 to 8 hours. The experiments were repeated 3-4 times and the results of such experiments are shown in table (1).

Experiment 2:

Safety and potency of BPL inactivated La Sota strain:

A batch form the vaccine was prepared (La Sota strain inactivated with 0.2% BPL), and the following tests were done on it.

A) Safety tests:

1. The virus after inactivation procedure (before being mixed with adjuvant) was inoculated in 25 eggs of 10 days old using a dose of 0.2 ml/ embryo. Mortality was recorded and HA test were done on egg fluids of individual eggs. Three blind passages were done before the judgement was reached.

RESULTS

Table (1): The influence of B-propriolactone on the infectivity and haemagglutinating activity of NDV.

	Velogenic strain (V)			Mesogenic strain (M)			Lentogenic strain (L)			Lentogenic strain (B_1)		
	0.2‰ BPL	0.05‰ BPL	0.01‰ BPL	0.2‰ BPL	0.05‰ BPL	0.01‰ BPL	0.2‰ BPL	0.05‰ BPL	0.01‰ BPL	0.2‰ BPL	0.05‰ BPL	0.01‰ BPL
	EID_{50} HA	EID_{50} HI	EID_{50} HI	EID_{50} HI	EID_{50} HI	EID_{50} HI	EID_{50} HA	EID_{50} HI	EID_{50} HI	EID_{50} HA	EID_{50} HI	EID_{50} HI
Original	8.0 9.0	8.0 8.0	8.0 8.7	8.3 9.0	8.6 9.3	9.3 9.2	9.0 9.2	9.3 9.0	9.0 8.7	8.5 9.0	8.5 9.1	9.0
Time 0	7.1 9.0	7.0 8.0	8.0 6.0	8.3 6.7	8.6 8.0	8.0 8.7	9.0 8.0	8.3 7.2	9.0 6.4	8.2 6.7	8.5 7.0	8.0
0.5 hours	6.2 8.6	5.4 7.2	5.4 7.2	8.0 4.4	8.0 7.3	8.0 6.2	8.0 6.7	8.3 6.4	8.8 4.0	7.2 4.3	7.5 5.9	8.0
1.0 "	5.3 8.0	6.8 6.5	4.5 6.5	2.4 7.3	3.3 7.6	6.6 8.0	4.7 8.0	5.5 7.6	5.8 8.5	2.0 6.5	2.9 6.7	8.0
1.5 "	4.5 7.7	3.8 6.5	4.1 6.5	1.3 7.3	2.3 7.6	5.6 8.6	3.1 7.6	4.5 7.6	4.6 8.3	zero 6.2	1.4 6.7	8.7
2.0 "	3.6 7.5	2.9 6.5	3.7 6.2	0.0 8.6	1.2 7.8	4.7 8.0	1.8 7.0	3.4 7.0	3.7 8.0	6.2	0.0 6.7	8.2
2.5	3.4 7.0	2.1 6.2	3.2 6.2	zero	7.0	4.0 8.0	zero 7.0	1.5 7.0	2.8 8.0			8.3
3.0 "	1.2 6.5	1.3 6.0	2.8 6.0			3.2 8.0	zero	zero 6.6	1.8 8.0			8.3
4.0 "	zero 6.0	0.5 6.0	1.9 6.0			1.2 7.6		6.6	zero 7.0			zero 8.0
4.5 "		zero	6.0 1.6	5.7		0.8 7.8			6.6			7.5
5.0 "			6.0 1.0	5.7		zero 7.8						
6.0 "			zero	5.7		7.0						

(8.1) : The figures under the LD_{50} and HA test represent average number of trials made

- HA = standard quantitative haemagglutination test
- EID_{50} = Embryo infectious dose fifty
- BPL = Beta propiolactone

Table (2): Clinical and Immunological response of chickens vaccinated with BPL - inactivated NDV

Weeks post inoculation	Clinical Response			Serological response									
	Apparently healthy No.	Affected No.	Dead No.	Distribution of HI - titers (Log_2) 0	1	2	3	4	5	6	7	8	9	GM Log_2
0	10	0	0	10	0	0	0	0	0	0	0	0	0	0
1	10	0	0	0	3	2	3	2	0	0	0	0	0	2,1
2	10	0	0	0	0	0	3	1	4	2	0	0	0	3,1
3	10	0	0					2	1	4	2	1	0	5.8

Vaccinated birds received 0.5 ml dose per bird (equevalent to approx. 10 4.8 EID_{50}). The vaccine was non-adjuvated to test the safety in 5 weeks old chicks

a) 4 HA units of NDV were used
HI = Hemagglutination - inhibition test

b) GM = Geometric mean

Table (3): Serological response of chickens vaccinated with 0.2% BPL - inactivated oil adjuvant NDV vaccine*

Weeks P.V.	birds N.	HI-test a) Distribution of HI - titer (log_2)													GM b) Log_2
		0	1	2	3	4	5	6	7	8	9	10	11	12	
0	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	40	6	10	11	10	3	0	0	0	0	0	0	0	0	1.8
2	40	0	0	3	7	13	12	4	0	0	0	0	0	0	4
3	40	0	0	0	0	1	3	7	17	6	6	0	0	0	7
4	20	0	0	0	0	0	1	4	8	5	2	0	0	0	7.1
5	20	0	0	0	0	0	2	8	8	2	0	0	0	0	6.4
6	20	0	0	0	0	0	0	11	9	0	0	0	0	0	6.4

* Vaccinated birds received a single intramuscular dose (0.5 ml/bird) at 7 weeks of age

a) 4 HA units were used

HI = hemagglutination - inhibition test

b) GM = Geometric mean.

Table (4): Protection efficiency against challenge with NDV in chickes vaccinated with 0.2% BPL - inactivated NDV vaccine

Time of chall a) in weeks post vaccination	No. of birds	N. of survivors	No. of dead birds b)	% of protection	HI titre GM c)
3	20	20	0	100 %	8.5
6	20	18	2	90 %	8.7
Control unvaccinated	10	0	10	0	0.0

Vaccinated as well as unvaccinated control birds were challenged with the VVNDV 3 weeks post vaccination. Another group of vaccinated chicks were challenged after 6 weeks post vaccination

a) chall = challenge of birds vaccinated by oily vaccine at 7 weeks old

b) Protection % = $\frac{\text{N. of survivors}}{\text{Total N. of challenged birds}} \times 100$

c) GM = Geometric mean

No egg mortality or positive HA activity was recorded, denoting that no residual virus infection was detectable.

2. Ten susceptible chicks 5 weeks old were inoculated with 0.5 ml. of the inactivated egg fluid per bird. Birds were kept under close observation for clinical manifestations and/or for 3 weeks. Blood samples were collected weekly and used in the HI test.

Results:

As shown in table (2).

3. Potency tests:

Unvaccinated susceptible chicks-forty chicks, 7 weeks were used in this study. They were inactivated with the BPL-inactivated oil adjuvant

vaccine prepared from La Sota strain. Each bird received 0.5 ml. subcutaneous in the dorsum of the neck.

Birds were kept under observation for 3 or 6 weeks. Blood smaples were collected weeekly and sera were used in the HI test to measure NDV-specific antibodies.

Three weeks after vaccination, twenty birds were taken for challenge, while the remaining birds were challengea, while the remaining birds were challenged 6 weeks post vaccination. Challenge was done using the VVNDV inoculated I/M 10^6 EID_{50}/bird.

Challenged birds were kept in a separate room under close observation. Morbidity and mortality was recorded daily. Blood samples were taken weekly and sera were tested in the HI test. Dead birds were necropsied for organs pathological changes. Liver, spleen and caecal tonsils were prepared for virus reisolation by egg inoculation.

RESULTS

1. Results obtained by the HI test for sera of vaccinacted birds were shown in table (3).

2. Results of challenge done on birds 3 and 6 weeks post vaccination as well as control unvaccinated birds were shown in table (4).

DISCUSSION

Newcastle disease virus is one of the mose important and serious pathogens causing heavy losses in poultry forms in Egypt. There is no doubt that the only means for controlling such a viral disease is by effective protection of birds using suitable vaccines.

Killed virus vaccine will be counteracted the problem of egg transmitted pathogens through live vaccines. The inactivation curves for the 4 strains of NDV, VVNDV, Komarov, LaSota and B1 with different concentration of B-propiolactone are shown in table (1) concluded that the lower concentration of BPL (0.01%) had no advese effect on NDV antigenicity, such findings agree with **Legnhausen et al. (1959)**. The La Sota strain has been selected for preparation of the vaccinal batch to be tested.

The inactivated vaccine was mixed with equal amount of Fruend's adjuvant and tested for its immunigenicity in 7 weeks old susceptible chicks. The humoral response was followed for 6 weeks, where a peak of HI antibodies was reached between 3rd to 4th week P.V. with titre of 7.1 then started to decline slowly.

In spite of that, it has to be known that the immune response achieved here is still below the level expected. We believe that this is mainly due to the absence of priming by a live virus, which is essential for reaching the optimal immune response **(Box and Furminger, 1985; Stone et al., 1981; Giambrone and Ronald, 1986)**. Evaluation of the protection capacity of the vaccine by the challenge test done at 3 and 6 weeks P.V. showed clearly that this protection was 100% and 90% respectively. Although vaccine have passed the acceptable level of protection for an inactivated vaccine **(Nedeciu, 1973; Stone et al., 1983 and Cardona et al., 1987)**.

The potency of the killed oil adjuvant vaccine prepared in this work from La Sota strain in protect-

ing chicks for a duration lasting up to 6 weeks in supported by the finding reported by **Duchatel and Vindevogel (1986)**, who proved the potency of killed La Sota strain to protect pigeons against virulent NDV infection even when the vaccine was given non adjuvant.

BPL was used because it has no deletarious effect on viral proteins (antigens) while its effect is mainly directed to viral nucleic acid (NA) reducing rapidly and drastically viral infectivity, as has also been found by **Keeble and Wade (1963), Cherby (1967) and Song and Lee (1988).**

The use of Tween 80 has main functions; the first is its usefulness as a food emulsifier, its capability in augementing the immune response to NDV antigen such properties agree with findings reported by **Rossi and Giusepp (1969)** who found that Tween 80 could enhance the mechanism of vaccinated chicks by raising the HI response.

In conclusion, it is quite clear that all tested parameters denotes that the BPL vaccine can be considered valid for vaccinating chicken against NDV infection.

REFERENCES

Ahmed, H.N. and Sabban, M.S. (1965): Studies on Newcastle disease vaccine in Egypt. Preparation and application of dissicated whole embryo vaccine. J. Arab. Vet. Med., 25: 165-180.

Allan, W.H.; Lancaster, J.E. and toth, B. (1973): The production and use of Newcastle disease vaccines. P. 35 Food and Agriculture Organization, Rome, Italy. 1115 pp.

Box, P.G. and Furminger, I.G.S. (1985): Newcastle disease antibody levels in chickens after vaccination with oil emulsion adjuvant killed vacine. Vet. Rec., 96:108-111.

Cardona, H.R. De, Robin, O.; Parva, L.G. (1987): Development and evaluation of an inacivated vaccine in oily adjuvant against Newcastle disease. Revista du Medicina Veterinariay de Zootecniq (1986, publ 1987), 39 (1-2) 5-13.

Cherby, J. (167): Immunization of fowls against Newcastle disease. 2- Innocuity and immunogenicity of the chemically inactivated virus. Rec. Med. Vet., 143, 647.

Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R. H. A. (1975): Medical Microbiology, The practice of Medical Microbiology, Twlfth Ed. Vol. 11.

Doubney, R. and Mansi, W. (1947): The occurrance of Newcastle disease in Egypt. J. Comp. Path., 58, 189.

Duchatel, J.P.; Vindevogel, H. (1986): Assessment of vaccination of pigeons against paramyxovirus type 1 infection with inactivated aqueous suspension or oil-emulsion vaccines. Avian Pathology, 15 (3), 455-465.

Eissa, J.M. (1960): Poultry diseases in southern region of U.A.R. 1st Ann. Vet. Cong. Egypt. Vet. Med. Ass., p. 19.

El-Salad, V.A. (1984): Studies on Immunization programmes adopted in commercial chicken flocks against Newcastle disease. Ph. De. Thesis, Fac. of Vet. Med., Alexandria University.

Fabricant, J. (1956): A modified chick embryo inoculation technique for the isolation of viruses from the respiratory tract of chickens. Proc. 28th Ann. Meet. N.E. Conf. Lab. workers pullorum Dis. Contr. Newark, Delare: 19-20.

Glambrone, J.J. and Ronald, P. Clay (1986): Vaccination of day-old broiler chicks against Newcastle disease and Infectious Bursal disease using commercial live and/or inactivation vaccines. Avian Diseases, 30 No. 3, 557-561.

Keeble, S.A. and Wade, J.A. (1963): Inactivated ND vaccines. J. Comp. Path., 73, 186-200.

Legenhausen, D.H.; Sinkiewicz, R.J. and Sullivan, J.E. (1959): Cited by J.E. Lancaster: Newcastle disease control by vaccination. Vet. Bull., Vol. 34 No. 2 (1964).

McDaniel, H.A. and Osborn, J.S. (1973): Diagnosis of velogenic viscerotropic Newcastle disease. J. Am. Vet. Med. Ass., 163: 1057-1070. Methods for Examination of Poultry Biologics for Identifying and Quantifying Avian Pathogens. National Academy of Sciences, Washington, D.C. (1971).

Nedelcium D. (1973): Inactivation of NDV with propiolactone studies of inactivation condition (concentration temperature and time). Lucrarile Institutuide Carcetari Veterinare Si Biopreparate (Pasteur, 167-177).

Reed, L.J. and Muench, H. (1938): Simple method of estimating fifry percent end points. Amer. J. Hyg., 27: 493-497.

Rossi, G.A. and Di Giuseppe, F. (1969): Vaccination against Newcastle disease with a virus grown in BHK-21 cell culture and inactivated with beta propiolactone.

Schmidt, N.J. and Lennette, E.H. (1967): Methods in immunology and immunochem istry., Vol. 1, 87, by William, C.A. and chase, M.W., Academic press, New York and London.

Sheble, A. (1960): Present situation of Newcastle disease in Egyptian region, U.A.R. Proc. 1st An. Vet. Cong. Egypt. Vet. Med. Ass., Cairo, p. 165.

Sheble, A. and Reda, M.I. (1978): Cited by Khafagy, A.K. (1972): Thesis M.V.Sc., Fac. Vet. Med., Cairo University.

Song, C.S.; Lee, T.C. (1988): Effects of chemical inactivation on virual polypeptides of Newcastle diseasevirus. Research Reports of the Rural Development Administration, Veterinary, 30 (3), 77-89.

Stone, H.D.; Brugh, M.; Beard, C.W. (1981): Comparison of three experimental inactivated oil-emulsion Newcas-

ile disease vaccines. Avian Diseases, 25 (4), 1070-1076.

Stone, H.D.; Brugh, M.; Beard, C.W. (1983): Influence of formulation on the efficacy of experimental oilemulsion Newcastle disease vaccines. Avian Dis., 27 (3): 688-697.

Zaher, A.; Eissa, Y.M. and Nafie, E. (1962): Immunization of baby chicks with B_1 and F strain of Newcastle disease vaccine. Proc. 3rd Ann. Vet. Congr. Egypt. Vet. Med. Ass., p. 231.