Studies on the Immunogenic Properties of La Sota NDV Propagated in Embryo Lung Primary Cells Culture

Moghaddam pour M., Tavasoli A. and Ebrahimi M.M.
Poultry Department, Razi-Vaccine & Serum Institute, P.O.Box 11365/1558, Tehran, IRAN.

Summary
Three passages of La Sota lentogenic Newcastle disease virus (NDV) in embryo lung cells culture caused changes in virulence and produced good cytopathic effect (CPE). Subsequently this refreshed La Sota strain was used for mass production of NDV vaccine. The results of potency and safety tests showed a significant increase in titer and immunogenicity of refreshed NDV vaccine strain without any great change in pathogenicity, intracerebral pathogenicity index (ICP), intravenous pathogenicity index. (IVPI)).

Keywords: Newcastle disease virus, La Sota strain, Embryo lung cells culture, Immunogenicity, Pathogenicity.

Introduction
Newcastle is known as an important disease in the world. It is classified into three different pathotype: lentogenic, mesogenic and velogenic strains. Some of virus characteristics is genetically determined by the virus strain but it is also largely dependent on the host cell (Nishikawa *et al.* 1983).
A NDV seed vaccine after storage at -60°C for a long time must be refreshed. FAO (Food and Agriculture Organization) standard procedure booklet recommends three passages of seed virus should be done in SPF (specific pathogens free) chicken (Avery and Niven 1971). Slosaris *et al.* (1989) made serial passages of two lentogenic NDV strains in kidney cell lines to increase their virulence and changed their viral biological propertis. In this report serial passages of La Sota strain in embryo primary lung cells culture was carried out and compared with La Sota NDV vaccine grown in embryonated eggs.

Materials & Methods

Virus: A lentogenic NDV strain (La Sota) was used in this study. It was harvested from infected amnio-allantoic fluid (AAF) of embryonated eggs. The titer was $10^{9.5}$ EID 50/ml.

Preparation of La Sota virus suspension: Three times passages in embryonated eggs with limiting dilution produce can be considered genetically pure and free from contaminants.

Primary cells culture: Monolayers of embryo lung cells culture were prepared from 13 day old SPF (Lohman company) embryonated eggs. The primary cells culture were grown in MEM (Minimum Essential Media) with 10% fetal calf serum (FCS) inactivated at 56°C for 30 min, and antibiotics (streptomycin 100μg/ml & penicillin 100U/ml). Cell cultuers were cultivated at 37.5°C in closed glassed.

Inoculation of cell cultures: In the first passage a 10^{-4} dilution of NDV La Sota strain was inoculated in cells culture. After 72-96 hrs cell culture flasks were frozen at -20°C. The infected cells were disrupted by 15 times freeze and thawing. Cell debris were pelleted by centrifugation at 4000 rpm for 15 min. The second and third passage was carried out in the same way.

Infectivity assay: NDV suspension prepared by refreshed strain was titrated in the embryonated eggs and the EID 50 was determined by the method of Reed and Muench (1938).

Intracerebral pathogenicity index (ICPI): 10 one-day old chicks devoid of NDV maternal antibody were injected intracerebrally with 50μl of new NDV suspension of 10^{-1} dilution. Chicks were observed for 8 and 12 days and scored for index calculation according to clinical symptoms and death.

Intravenous pathogenicity index (IVPI): 10 six-week old chicken devoid of NDV antibody were injected intravenously with 0.1 ml of new virus suspension of 10^{-1} dilution. The birds were observed daily for 10 days and scored for index calculation according to clinical symptoms, paralysed and death.

Production of the master seed of NDV vaccine (La Sota strain): After three successive passages on lung cells culture, the content of virus was inoculated in embryonated eggs of 10^{-1}-10^{-2}. Then 8 passages was conducted in embryonated eggs at 10^{-4}-10^{-3} dilution. The last passage was harvested as master seed

NDV and then working seed was produced. In all inoculations, 10 day old embryonated SPF eggs were used.

Serological response - Haemagglutination inhibition test (HI): 25-3 week old susceptible chicken were inoculated with one dose (EID 50 = 6.10^6) by ocular route. After 3 weeks blood samples were collected and HI test was conducted using round-bottomed microplates in conventional method.

Potency test: 25 vaccinated along with 10 SPF chicken as control were injected with 500μl (ELD 50=10^5) of velogenic NDV strain isolated from Karaj area by intramuscular. They were observed for 10 days. The properties of velogenic NDV strain summerized in Table 1.

Table 1: Properties of velogenic NDV strain.

Test	ICPI (8day)	*ELD 50 (log 10)	* CLD 50	*MDT (hr.)
Velogenic NDV strain	2.3	6	9.47	38.4

CLD 50 : Chicken lethal dose 50
MDT : Mean death time
ELD 50 : Embryo lethal dose 50

Results

Propagation in lung cells culture: Following inoculation with the lentogenic strain, a cytopathic effect (CPE) was observed and in the first passage a weak HA activity was recorded.

Pathological assay (ICPI , IVPI): After 3 times passage, new La Sota strain showed a good titer but without any problem in ICPI & IVPI. The results are summerized in Table 2.

Table 2: Pathological assay after 3 times passage

Test	ICPI (8day)	ICPI (12 days)	IVPI	EID 50 (log 10)
NDV strain	0.3	0.37	0	7.5

Table 3: Average titer of sera sample of 25 chicken following one dose of new La sota vaccine compared with market La Sota vaccine Challenge of 100% of vaccinated chicken with new La Sota vaccine provided full protection.

Test	NDV strains	
	*A	*B
EID 50 (log 10)	10.58	10.09
HA (log 2)	7.64	7.32
HI (log 2)	4.2	4.25

A . New La Sota vaccine

B . Market La Sota vaccine

Discussion

The various strains of NDV are classified as lentogenic, mesogenic and velogenic according to their virulence and pathogenicity in chicken. Infection in fowl ranges from inapparent to severe and ultimately fatal, involving various organs (Slosaris *et al.* 1989). It has been reported that there is no correlation between antigenic and virulence (Reed and Muench 1938, Levy *et al.* 1975, Pennington 1978). In addition, the host cell might influence viral characteristics through insertion of such different components as glycoproteins, lipids, and carbohydrates into the viral membrane (Allan 1971).

Allan in 1971 had suggested a procedure to refresh master seed NDV in which it is necessary to make three passages in SPF chicken *(in vivo)*. In this study embryo lung cells culture was used. In order to refresh master NDV strain usage of embryo lung cells culture *(in vitro)* proved to be safer and more available as shown by a number of techniques. This method could increase the immunogenicity and no change in pathogenicity of NDV La Sota strain.

Acknowledgements

We would like to thank Dr. MH. Ansarifard and Dr. SA. Pourbakhsh for their advices and helpful discussions. We thank Mr. R Hosseini, M Alikhani, E Barband and MA Afkami for their technical assistance.

References

Allan WH. (1971). The problem of Newcastle disease. Nature 234:129-131.

Avery RJ, Niven J. (1971). Use of antibodies to purified Newcastle disease virus glycoproteins for strain comparisons and characterization. Infect. Immun. 26(3):795-801.

Levy R, Spira G, Zakay-Rones Z. (1975). Newcastle disease virus pathogenesis in the respiratory tract of local or systemic immunized chickens. Avian Dis. 19:700-706.

Nagai Y, Klenk HD Rott R. (1976). Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. Virology. 72:494-508.

Nishikawa K, Isomura S, Suzuki S, Watanabe E, Hamaguchi M, Yoshida T, Nagai Y. (1983). Monoclonal antibodies to the HN glycoprotein of Newcastle disease virus. Biological characterization and use for strain comparison. Virology. 130:318-330.

Pennington TH. (1978). Antigenic differences between strains of Newcastle disease Virus. Arch. Virol. 56:345-351.

Reed LJ, Muench HA. (1938). A single method of estimating fifty percent endpoints. Am J. Hyg. 27:463-497.

Slosaris M, Levy B, Katz E, Levy R, Zakay-Rones Z. (1989). Elevated virulence of Newcastle disease virus strains following serial passages in kidney cells in vitro. Avian Dis. 33:248-253.

Waterson AP, Pennington TH, Allan WH. (1967). Newcastle disease virus a preliminary study. Br. Med. Bull. 23:138-143.