

NOTE

Immune response of chickens to four lentogenic strains of Newcastle disease virus propagated in lamb kidney cell cultures

by

A. TAVASSOLI

SUMMARY

Four lentogenic strains of Newcastle disease virus (NDV) were compared for their immunogenicity and pathogenicity in chicken. These strains were also compared for their pathogenicity in the Lamb Kidney cell cultures.

The lasota and F strains gave slightly better protection than B1 and TCND strains when immunity was challenged with virulent Newcastle virus. The lasota strain caused slightly greater respiratory reaction and greater potential for spreading from chickens than the B1 strains. Serological tests, (HI) before and after vaccination, showed that there was no spreading of TCND vaccine virus to unvaccinated contact controls which were kept in the same unit. This finally confirms the data obtained by Bankowski (3,4,6).

INTRODUCTION

After the discovery of the B1 strain, many other lentogenic strains have been identified such as the lasota and the F strains. The F strain isolated by the Asplin (1952) and the five newly isolated strains by Beaudette and Hudson (1956), these latter strains have frequently been referred to as B1 type strains (7).

Hanson et al in a comparison of the immunogenicity and pathogenicity of five strains of Newcastle Disease Virus (NDV) as formalized vaccines showed that chickens immunized with Manhattan-Kansas and Roakin-NJ, were more resistant to challenge than chickens receiving GB Texas, Roakin and B1 strains.

Sulvin et al demonstrated that a single injection of GB Texas established a better protection than a single injection of Roakin or Manhattan-Kansas. (8)

Winterfield et al (1957) demonstrated that the lasota strain, when administered through the drinking water, gave a better response than the B1 and F strains. (9)

The purpose of this study was to determine the degree of susceptibility of Lamb Kidney cell cultures for the four lentogenic strains of Newcastle Diseases Virus and to compare their pathogenic and immunogenic properties in chickens.

MATERIAL AND METHODS

Virus:

The five following strains of NDV were used throughout these experiments. Four lentogenic strains were used such as the NJ-lasota, the F-Asplin, the B 1-Hitchner, the Bankowski-TCND and one velogenic strain Zabol which was isolated in Razi Institute, by Sohrab and Baharsefat from tissue of sick chickens.

This velogenic strain was used for challenge. The challenge dose given by intramuscular route in all cases, was 1,000,000 ELD 50.

CELL CULTURE:

Primary lamb kidney (LK) cell cultures were prepared in Leighton tubes. The growth medium consisted of Earl's Medium with Yeast and Lactalbumine hydrolysate (EYL) with 10% calf serum.

CHICKENS:

The white leghorn chickens were obtained from Poultry Dept. of Live stock Institute, Tehran, the flock had not been vaccinated against any disease. These chickens divided in 3 groups as follows:

GROUP 1) Which consisted of 4 subgroups of 100 chickens each were inoculated with 0.5 ml. ($10^{6.2}$ per 0.1 ml.) of four vaccines (NJ-Lasota, F-Asplin, B 1-Hitchner and TCND-Bankowski) which propagated in Lamb Kidney Cell monolayers.

GROUP 2) which consisted of 4 subgroups of 25 chickens each, which were used as contact controls. Each group of vaccinated chickens with their contact controls were held in a separate isolated unit.

GROUP 3) Which consisted of 50 unvaccinated chickens was held in another isolation unit and used as challenge control at the end of the experiments.

Preparation of Vaccines

Four strains of Newcastle Disease Virus were adapted and grown in Lamb K. Cell Cultures up to 17 passages. Supernatant fluids of the infected cultures were stored at -20°C . and were used as vaccine.

RESULTS

Results of the cytopathogenic effect (CPE), hemadsorption and staining with acridine orange (A.O) of four strains of N.D. Virus (B 1, TCND, F and Lasota) propagated on lamb kidney cell culture after 24,48,72 and 96 hours are summarized in Table 1.

1-24 hours after inoculation:

One plus CPE and one plus hemadsorption was observed (using 0.4% chicken red blood cell in the buffered saline) with B 1, Lasota and F strain and no CPE and hemadsorption with TCND strain during these observation periods 2-48 hours after inoculation: Syncytium, hemadsorption in lamb kidney cell culture were observed with four strains.

3-72 hours after inoculation: Hemadsorption, Karyorrhexis of nuclei in syncytia and hyperplasia were observed with the four strains.

4-96 hours after inoculation: The CPE, hemadsorption and hyperplasia increased 4 plus CPE and 4 plus hemadsorption were observed by the Lasota, F and B 1 strains.

TCND strain showed 3 plus CPE and 3 plus hemadsorption. However the Lasota strain showed more pathogenic effect than F, B 1 and TCND in lamb kidney cell cultures.

TABLE 1
Results of C P E, hemadsorption and staining with A.O¹. after 24,48,72 and 96 hours at 17th passage level.

		Observations							
		24 Hours		48 Hours		72 Hours		96 Hours	
Cell culture	Virus strains	CPE	A.O. & Hd2	CPE	A.O. & Hd2	CPE	A.O. & Hd2	CPE	A.O. & Hd2
Lamb kidney cell cultures	N J-Lasota	+	+	++	++	+++	+++	++++	++++
	F-Asplin	+	+	++	++	+++	++	++++	++++
	B1-Hitchner	+	+	++	+	+++	+++	++++	++++
	TCND-Bankowski	-	-	+	+	++	++	+++	+++

1 = A.O. =Acridine orange

2 = Hd =Hemadsorption

ESTIMATION OF 50 END POINT IN EMBRYONATING EGGS

A pool of 72 and 96 hours of the each supernatant fluids of the cultures was prepared and titrated in 9 day old chicken embryonating eggs.

Each of the supernatant fluid was clarified by centrifugation and 0.1 ml. of each fluid were inoculated into the allantoic cavity of 9 day old chicken embryonating eggs (CEE), slight hemorrhages were found in the dead embryos infected with F, B 1 and TCND strains, but the reaction was more with Lasota strain.

As shown in Table 2, the Lasota strain produced higher titer ($10^{6.6}$ ELD₅₀ per 0.1 ml.) and the lowest infectivity titer was obtained with the TCND strain propagated, in L.K. Cell Culture ($10^{6.2}$ ELD₅₀).

The hemagglutination (HA) activity of allantoic and amniotic fluids (aaf) in the dead embryos with the four strains was positive.

IMMUNIZATION OF CHICKENS:

Each of the titrated vaccine was diluted to $10^{6.2}$ ELD₅₀ to contain equal concentration. Each chicken in each subgroup of group one received 0.1 ml. equal concentration. Each chickens in each subgroups of group one received of each vaccine with ELD₅₀ of $10^{6.2}$ per 0.1 ml. respectively.

Hemagglutination-Inhibition (HI) antibody titers:

1-HI titer before vaccination:

TABLE 2
Titration of the four strains of NDV propagated in Lamb kidney cell culture in chicken embryonating eggs.

Cell culture	Virus strains	Titer ELD 50 per 0.1 ml.
Lamb kidney cell cultures	NJ-Lasota	10 ^{6.6}
	F-Asplin	10 ^{6.4}
	B 1 -Hitchner	10 ^{6.3}
	TCND-Bankowski	10 ^{6.2}

All of the birds were bled to determine the presence of HI antibodies (degree of passive immunity) before vaccination when they were 23 days old. None of the birds had any detectable HI antibodies.

II-HI Titer in chickens vaccinated with the four virus vaccines propagated in L.K. cell Cultures, were as follows:

- 1—The birds vaccinated with NJ-Lasota propagated in L.K. cell culture showed a high titer by hemagglutination-Inhibition test. The HI titer (GMT) was 140 (Fig. No 1 and Table 3).
- 2—The results of HI (GMT) response in chickens vaccinated with F. Asplin propagated in Lamb Kidney cell culture was 130.
- 3—Chickens immunized with B 1 and TCND virus vaccines showed 125 and 120 GMT respectively.

TABLE 3

Serological response of four groups of chickens of 30 days old vaccinated with four strains of ND viruses propagated in LK and response of the chickens to intramuscular challenge at 9 weeks of age, (1,2,5). *

HI Titer -GMT

Sub group N° **	N° of birds	Before vaccination at 23 days	4 weeks following Vaccination	% Survival after challenged at 9 weeks/age
1	100	0	140	100
2	100	0	130	100
3	100	0	125	100
4	100	0	120	100
5	25	0	0	0
6	20	0	0	0

* All chickens were resistant to challenge with 1,000,000 doses ELD₅₀ of strain ZABOL N.D. Virus.

- ** 1— Inoculated with NJ-Lasota strain
2— " " F-Asplin "
3— " " B 1 Hitchner Strain
4— " " TCND Bankowski strain
5— Contact control
6— Challenge control.

Challenge:

30 days after vaccination all the subgroups containing vaccinated chickens together with contact controls and the group of unvaccinated control chickens were challenged with Zabol strain.

The challenge dose of Zabol virus for each bird consisted 1 ml. of a virus suspension diluted to contain 1,000,000 doses of ELD₅₀ (1 ml. of 10⁻⁵ ELD₅₀). The results were as follows:

1—All chickens vaccinated with 0.5 ml. of four vaccines resisted the injection, virulent Zabol virus. Table 3.

2—All of contact controls and unvaccinated control chickens died within 9 days post infection. Table 3.

Conclusion:

At the present the lentogenic strains of N.D.V. are being used more than mesogenic and velogenic strains in production of N.D. vaccine.

The cytopathic effect (CPE), hemadsorption and reactions of acridine orange staining were similar with all strains on cell culture but the time of appearance of the reactions slightly varied one from another (Table 1).

The infectivity titer of the four strains in chicken embryos varied and titers of virus increased in the following order: NJ-Lasota, F-Asplin, B1 Hitchner and TCND-Bankowski strains (Table 2).

Vaccines consisted of the supernatant fluids of the infected cultures. The titer of four different vaccines were adjusted so that each contained 10^{-6.2} ELD 50.

Among the four groups of vaccinated chickens the Lasota and F vaccines caused depression, slight rales and two cases of paralysis with Lasota vaccine were also observed. The birds vaccinated with B1-Hitchner and TCND-Bankowski vaccines did not show any clinical signs of the disease during a 4 weeks observation period following vaccinations.

4 weeks after vaccination all birds were challenged intramuscularly with 1 ml. of virulent strain of Zabol virus.

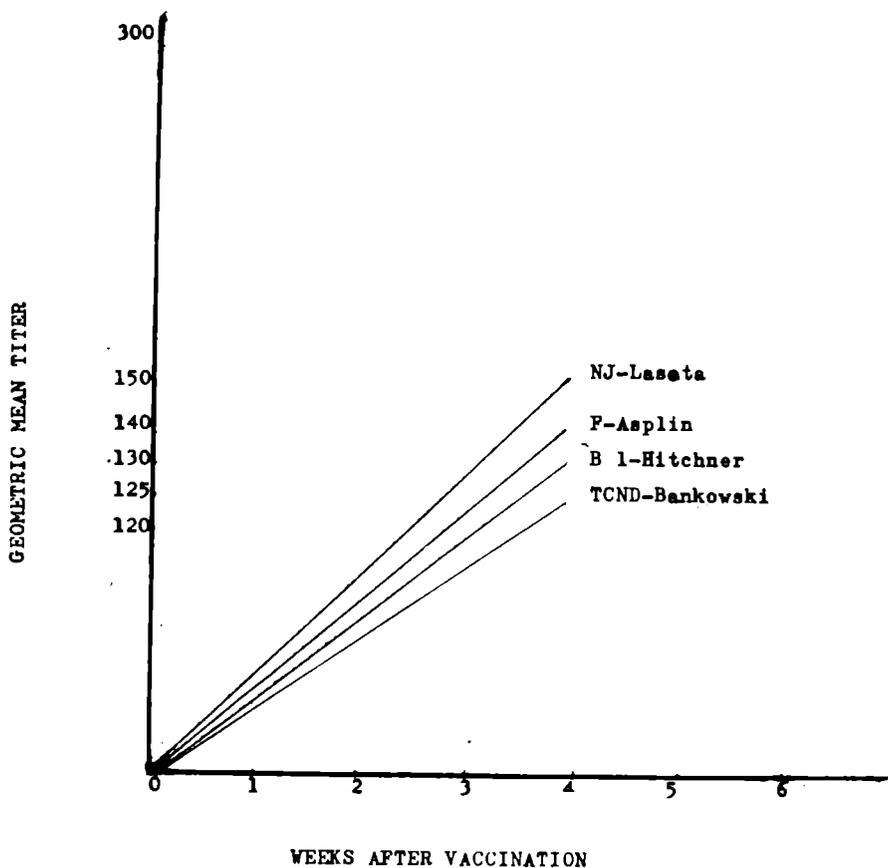
All of the contact controls and unvaccinated control chickens died after challenge with the virulent Zabol virus.

Vaccines prepared with four strains of (NJ-Lasota, B1-Hitchner, F-Asplin and TCND-Bankowski) in L. kidney cell cultures produced a good antibody titer in vaccinated chickens and consequently conferred a solid immunity in vaccinated birds.

Serological tests (HI) before and after vaccination revealed that there were no spreading of B 1 and TCND virus vaccines, but slight spreading (HI=1/10)

of Lasota and F vaccines occurred when contact controls were kept with immunized birds.

Fig 1 : HI titers (GMT) in 8 weeks old chickens 4 weeks after vaccination with four strains of N.D.V. propagated in lamb kidney cell cultures.



REFERENCES

- 1— Tavassoli, A & Bankowski, R.A.
Arch. Inst. Razi, 1970, 22 : 215-225.
- 2— Bankowski, R.A. & Kinjo, J.
Avian Dis, 1965, 9 : 157-170.
- 3— Bankowski, R.A. & Corstvet, R.
Avian Dis, 1962, 6 : 333-348.
- 4— Bankowski, R.A.
Avian Dis, 1957, 1 : 195-207.

- 5— Bankowski, R.A.
Proc. Soc. Exp. Biol. med., 1957, **96** : 114-118.
- 6— Bankowski, R.A. Gerlach, H. & Mikami, T.
Avian Dis, 1958, **12** : 217-259.
- 7— Hanson, R.P.
Newcastle Disease Virus, 1964, 85-98, Published by the University of Wisconsin
press, Madison, Wisconsin, 53701.
- 8— Change, P.W. Yates, V.J. Dardiri, A.H. & Fry, D.E.
Avian Dis, 1960, **4** : 384-390.
- 9— Winterfield, R.W. & Seadals, E.H.
Amer. J. Vet. Res. 1956, **17** : 5-17.