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NOTES

Plaque Assay for Avirulent (Lentogenic) Strains of Newcastle Disease Virus

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Avirulent (lentogenic) strains of Newcastle disease virus form plaques on chicken embryo lung monolayers in 48 to 72 h.

Newcastle disease virus (NDV) strains can be divided into three categories on the basis of virulence: lentogenic (avirulent), mesogenic, and velogenic (fully virulent) groups (3). The lentogenic strains lack neurovirulence and do not form plaques in chicken embryo fibroblast (CEF) cell cultures (4, 7). This can account for the scarcity of reported quantitative studies on lentogenic strains and for the fact that the few data available were derived almost exclusively from titrations in eggs (6).

This paper describes plaque formation by lentogenic NDV strains in chicken embryo lung cell (CEL) cultures. This simple, rapid and reproducible procedure proved to be helpful for the titration and plaque purification of such strains.

The lentogenic NDV strains LaSota, F, Ulster, and Queensland V4 were used. The origin and method of propagation of these strains were described earlier (5).

CEL cultures were prepared from 14-day-old chicken embryos. Lung cells were obtained by digestion in 0.1% trypsin (Difco) and were seeded in TC Medium 199 (Difco) containing 5% calf serum (derived from colostrum-deprived calves).

Cell monolayers were grown in petri dishes (Anumbra) 5 cm in diameter (3.5×10^6 cells per dish) and were used for titration after 20 to 24 h.

The overlay medium contained 0.85% Special Agar-Noble (Difco) purified with ethylenediaminetetraacetic acid at pH 7.0, 5% calf serum, 1% of a 5% sodium bicarbonate solution, and the usual concentrations of penicillin and streptomycin in TC Medium 199.

Plaque formation in CEL culture by the lentogenic LaSota, F, Ulster, and Queensland V4 strains is shown in Fig. 1. The plaques formed by lentogenic strains were 0.5 to 2 mm in diameter. A total of 14 lentogenic strains were examined, all of which formed plaques in CEL culture within 48 to 72 h. Embryonic chicken lung is a rich cell source, 45 to 55 $\times 10^6$ cells being obtainable from each pair of lungs, and the cell monolayer required for plaque assay is more easily reproducible than with kidney cells.

Barahona and Hanson (2) titrated lentogenic NDV strains in CEF culture in the presence of Mg^{2+} and diethylaminoethyl dextran. In our assay, five to ten times lower titers were regularly obtained with this method compared with titrations in CEL. Shingh et al. (8) used agarose in the overlay for the plaque titration of the

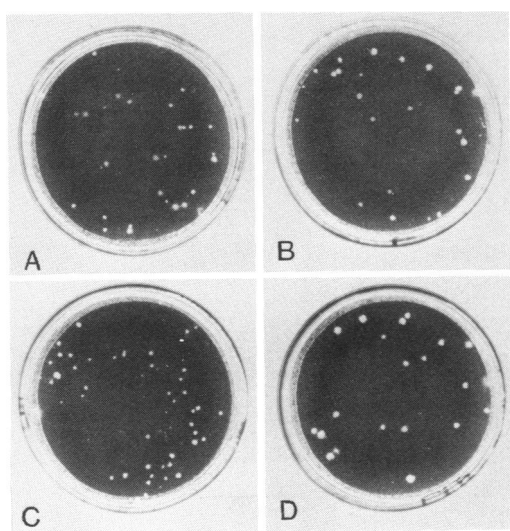

FIG. 1. *Plaque formation by lentogenic strains of NDV on chicken embryo lung monolayer. (A) LaSota; (B) F; (C) Queensland V4; (D) Ulster.*

TABLE 1. *Plaque counts of lentogenic strains of NDV in CEF and CEL*[a]

Cell	Overlay	NDV strain	
		LaSota	F
CEF	Agar	np[b]	np
CEF	Agar[c] + $MgCl_2$ + DEAE	160	57
CEF	Agarose	np	21
CEL	Agar	>800	195

[a] Monolayers were infected with 0.1 ml of 10^{-5} dilutions of virus samples, and plaques were counted after 4 days on CEF and 3 days on CEL.

[b] np, No plaques.

[c] 30 mM $MgCl_2$ and 200 μg of diethylaminoethyl (DEAE) dextran per ml were used.

lentogenic F strain and obtained plaques on the 4th to 5th day of incubation. It was found, however, that plaques formed in CEF cultures under agarose (Calbiochem) were turbid and not readable until 5 to 7 days after infection. Also, plaque counts were lower than in CEL culture, and certain strains had titers 2 to 3 logarithmic orders below the levels reached in CEL (Table 1).

Although the lentogenic NDV strains are capable of forming plaques in embryonic kidney cell culture (1), these cells, unlike CEL, may be prone to degeneration.

When samples of strains LaSota and Ulster were titrated in 9-day-old chicken embryos, values of 6.3×10^9 ($10^{9.8}$) and 2.5×10^9 ($10^{9.4}$) mean egg infective dose/ml were calculated, respectively; when the same dilutions of these strains were assayed in CEL, titers of 1.6×10^9 and 1.4×10^9 plaque-forming units/ml were obtained. Thus, plaque assay in CEL proved to be only 2 to 4 times less sensitive than in the egg system.

LITERATURE CITED

1. Bankowski, R. A. 1964. Cytopathogenicity of Newcastle disease virus, p. 231. *In* R. P. Hanson (ed.), Newcastle disease virus: an evolving pathogen. The University of Wisconsin Press, Madison.
2. Barahona, H. H., and R. P. Hanson. 1968. Plaque enhancement of Newcastle disease virus (lentogenic strains) by magnesium and diethylaminoethyl dextran. Avian Dis. **12**:151-158.
3. Hanson, R. P., and C. A. Brandly. 1955. Identification of vaccine strains of Newcastle disease virus. Science **122**:156-157.
4. Hanson, R. P., J. Spalatin, J. Estupian, and G. Schloer. 1967. Identification of lentogenic strains of Newcastle disease virus. Avian Dis. **11**:49-53.
5. Lomniczi, B. 1973. Studies on interferon production and interferon sensitivity of different strains of Newcastle disease virus. J. Gen. Virol. **21**:305-313.
6. Reeve, P., and A. P. Waterson. 1970. The growth cycle of avirulent strains of Newcastle disease virus. Microbios **2**:5-9.
7. Schloer, G. M., and R. P. Hanson. 1968. Relationship of plaque size and virulence for chickens of 14 representative Newcastle disease virus stains. J. Virol. **2**:40-47.
8. Shingh, K. V., Najla Saad, and A. El Zein. 1970. Sensitivity of the plaque technique for the study of selected vaccine strains and a virulent strain of Newcastle disease virus. Appl. Microbiol. **20**:638-640.