Preliminary Observations on the Pathogenesis of a Virulent Strain of Newcastle Disease Virus in Chickens

K. V. SINGH, NAJLA SAAD, AND A. EL ZEIN

Department of Virology, Fanar Regional Poultry Laboratory, Near East Animal Health Institute, Beirut, Lebanon

Received for publication 13 January 1971

Development of Newcastle disease, after experimental and natural infection with the virulent strain VLT of Newcastle disease virus, and its growth and distribution in some selected tissues as assayed by the enumeration of plaques are reported.

The plaque technique developed by Dulbecco (1) has been extensively used in studies of Newcastle disease virus (NDV; references 2, 6–8). Despite its potential value in yielding precise determination of virus, the technique was little utilized in studies of the pathogenesis of the disease in the domestic fowl or in epizootiological investigations.

Earlier workers (3, 4, 9) have reported successful isolation and enumeration of different strains of NDV in embryo eggs from various tissues after experimental or natural infection of chickens. In this paper we describe the development of Newcastle disease (ND) in chickens after experimental and natural infection and present data on proliferation of the virus in some selected tissues, as enumerated by the plaque technique.

The virulent strain VLT of NDV, isolated during 1968 from an outbreak of highly fatal ND among chickens at Talamara, Lebanon, was plaque purified and used in its third chick embryo passage level for these experiments. Virus stock was prepared by inoculating with approximately $10^4$ plaque-forming units (PFU) in 0.1 ml of seed virus into the allantoic cavity of 9-day-old chick embryos. Infected allantoic fluid was stored in 1-ml amounts at $-60^\circ$ C. Primary chick embryo cell cultures were prepared from minced 9-day-old decapitated chick embryos subjected to repeated trypsinization. The cells were grown in Eagle's minimum essential medium (MEM) containing 5% calf serum and 8% Tryptose phosphate broth. Approximately $5 \times 10^6$ cells per 2-oz prescription bottle were seeded, and monolayers were overlaid after infection with 6 ml of overlay medium, which consisted of Hanks balanced salt solution without phenol red, 0.5% lactalbumin hydrolysate, 1.0% Noble agar (Difco), 3% horse serum, 1.5% of 1:1,000 dilution of neutral red, 5% of 4.4% sodium bicarbonate solution, 100 units of penicillin and 100 $\mu$g of streptomycin per ml. Monolayers were used for virus assays 3 days after seeding (10). Seventy White Leghorn six-week-old chicks were divided into seven groups of 10 each. They were caged and placed in a room in which birds were never kept before. To avoid the risk of transmission of infection through feed and water supplies, each group was provided with its own feed and water. One group was maintained as contact controls, and the birds in the remaining groups were inoculated intramuscularly (pectoral muscles) with 0.5 ml of serial 10-fold dilutions of VLT strain of NDV. Chicks were observed twice daily. At postmortem examination, brain, spleen, trachea, and lung tissues were removed aseptically with separate, sterile instruments to avoid cross contamination from dead chickens as well as from sick ones killed by cervical dislocation. A 10% (w/v) suspension of tissue was made in MEM containing 1,000 units of penicillin and 1,000 $\mu$g of streptomycin per ml. Tissue suspensions were kept frozen at $-60^\circ$ C until tested. All end points were calculated by the Reed and Muench method (5).

The embryo-propagated virus stock had a titer of $10^9$ 50% chicken lethal doses per 0.5 ml, indicating that the virus strain is highly virulent for susceptible chickens. The incubation period varied from 3 to 4 days, but the majority of birds showed symptoms on the third day. Respiratory symptoms with railes were pronounced in almost all birds. Most birds also showed typical nervous symptoms. Death generally oc-
curred within 2 to 4 days after the onset of the symptoms. Torticollis and lateral movement of the head were commonly observed. A few birds developed paralysis of both legs. The gross pathological lesions consisted of extensive involvement of the proventricular submucosa and intestinal follicles. Severe hemorrhagic necrotic lesions adjacent to lymphoid plaques were also common. Infection spread easily to the contacts as signs appeared 4.8 days after probable exposure (Table 1).

A summary of virus titers in various tissues is presented in Table 2. The VLT strain multiplied extensively in the tested tissue, namely, spleen, trachea, brain, and lungs. Virus titers varied between $10^{3.5}$ and $10^{3.1}$ PFU/g of the brain tissue, indicating the relationship between concentration of virus in the brain and occurrence of nervous symptoms. Large amounts of virus were present in the tested tissues even on the day the chickens first showed symptoms, indicating that generalization of NDV in chickens probably occurred before the onset of clinical signs. Virus may therefore be excreted 1 or 2 days preceding the appearance of clinical signs. From the practical standpoint, it seems that probably the trachea is more suitable than other tissues for virus recovery. Bird 1848, which had a moderate quantity of virus in its trachea but not in the spleen, brain, or lung, remained apparently healthy, whereas its cagemates died on the sixth day. In bird 1844, which almost recovered after showing respiratory and nervous signs (paralysis of extremities), a high concentration of virus was found in the trachea and brain when killed on the 10th day. It is possible that such birds can become effective carriers.

From our study it can be concluded that the strain VLT is highly pathogenic for 6-week-old chickens. In our experience it spread readily and multiplied extensively in various tissues after experimental and natural infection. Critical organs, damage of which reflects the occurrence of symptoms, were the brain, trachea, and lungs. The virus seemed to generalize before the onset of clinical signs, and we consider that it could disseminate during this period. It has been observed that some birds can recover and may possibly become carriers after infection. The po-

| Bird no. | Virus dose (log 10 PFU) | Time of sacrifice PID | Condition* | Virus titer log 10 PFU/g of tissue |
|----------|------------------------|-----------------------|------------|----------------------------------|
|          |                        |                       |            | Spleen | Trachea | Brain | Lung |
| 1768     | 4.6                    | 4                     | D          | 9.3    | 8.9    | 8.1   | 8.9  |
| 1761     | 4.6                    | 5                     | D          | 6.8    | 7.6    |       |      |
| 1769     | 4.6                    | 4                     | M          | 6.9    | 7.9    | 6.4   | 7.4  |
| 1753     | 3.6                    | 5                     | M          | 7.0    | 7.8    | 7.4   | 8.3  |
| 1857     | 3.6                    | 5                     | M          | 6.6    | 5.3    |       |      |
| 1844     | 2.6                    | 10                    | P          | 5.7    | 6.5    |       |      |
| 1848     | 2.6                    | 10                    | N          | 0      | 2.9    | 0     | 0    |
| 1813     | 1.6                    | 6                     | M          | 7.5    | 7.4    | 8.5   | 8.1  |
| 1743     | 1.6                    | 6                     | M          | 6.4    | 7.5    | 5.9   | 7.4  |
| 1760     | 0.6                    | 5                     | D          | 8.1    | 7.2    | 6.3   | 7.6  |
| 1806     | cc*                    | 2                     | S          | 8.3    | 8.5    | 6.4   | 8.3  |
| 1809     | cc                     | 1                     | S          | 5.7    | 7.4    | 6.6   | 7.5  |
| 1810     | cc                     | 1                     | S          | 5.7    | 7.4    | 6.4   | 8.3  |

* Plaque-forming units.

† Postinoculation day.

* Condition of the bird at the time of sacrifice. Abbreviations: D, dead; M, moribund; P, paralyzed; N, normal; S, sick.

"Not tested.

# Contact control.

/ Days after signs appeared.

A summary of virus titers in various tissues is presented in Table 2. The VLT strain multiplied extensively in the tested tissue, namely, spleen, trachea, brain, and lungs. Virus titers varied between $10^{3.5}$ and $10^{3.1}$ PFU/g of the brain tissue, indicating the relationship between concentration of virus in the brain and occurrence of nervous symptoms. Large amounts of virus were present in the tested tissues even on the day the chickens first showed symptoms, indicating that generalization of NDV in chickens probably occurred before the onset of clinical signs. Virus may therefore be excreted 1 or 2 days preceding the appearance of clinical signs. From the practical standpoint, it seems that probably the trachea is more suitable than other tissues for virus recovery. Bird 1848, which had a moderate quantity of virus in its trachea but not in the spleen, brain, or lung, remained apparently healthy, whereas its cagemates died on the sixth day. In bird 1844, which almost recovered after showing respiratory and nervous signs (paralysis of extremities), a high concentration of virus was found in the trachea and brain when killed on the 10th day. It is possible that such birds can become effective carriers.

From our study it can be concluded that the strain VLT is highly pathogenic for 6-week-old chickens. In our experience it spread readily and multiplied extensively in various tissues after experimental and natural infection. Critical organs, damage of which reflects the occurrence of symptoms, were the brain, trachea, and lungs. The virus seemed to generalize before the onset of clinical signs, and we consider that it could disseminate during this period. It has been observed that some birds can recover and may possibly become carriers after infection. The po-

A summary of virus titers in various tissues is presented in Table 2. The VLT strain multiplied extensively in the tested tissue, namely, spleen, trachea, brain, and lungs. Virus titers varied between $10^{3.5}$ and $10^{3.1}$ PFU/g of the brain tissue, indicating the relationship between concentration of virus in the brain and occurrence of nervous symptoms. Large amounts of virus were present in the tested tissues even on the day the chickens first showed symptoms, indicating that generalization of NDV in chickens probably occurred before the onset of clinical signs. Virus may therefore be excreted 1 or 2 days preceding the appearance of clinical signs. From the practical standpoint, it seems that probably the trachea is more suitable than other tissues for virus recovery. Bird 1848, which had a moderate quantity of virus in its trachea but not in the spleen, brain, or lung, remained apparently healthy, whereas its cagemates died on the sixth day. In bird 1844, which almost recovered after showing respiratory and nervous signs (paralysis of extremities), a high concentration of virus was found in the trachea and brain when killed on the 10th day. It is possible that such birds can become effective carriers.

From our study it can be concluded that the strain VLT is highly pathogenic for 6-week-old chickens. In our experience it spread readily and multiplied extensively in various tissues after experimental and natural infection. Critical organs, damage of which reflects the occurrence of symptoms, were the brain, trachea, and lungs. The virus seemed to generalize before the onset of clinical signs, and we consider that it could disseminate during this period. It has been observed that some birds can recover and may possibly become carriers after infection. The po-
Potential value of the plaque technique in primary chick embryo cell culture for the study of pathogenesis of ND has been indicated.

These studies were jointly supported by the Fanar Regional Poultry Laboratory at the Agricultural Research Institute, Lebanon, and Near East Animal Health Institute, which is a United Nations Development Program/Special Fund Project administered by the Food and Agriculture Organization of the United Nations.

We thank J. Haraoui and J. Rizk for their continuous interest and encouragement throughout this investigation.

LITERATURE CITED

1. Dulbecco, R. 1952. Production of plaques in monolayer tissue cultures by single particles of an animal virus. Proc. Nat. Acad. Sci. U.S.A. 38:747.
2. Granoff, A. 1962. Heterozygosis and phenotypic mixing with Newcastle disease virus. Cold Spring Harbor Symp. Quant. Biol. 27:319-326.
3. Hofstad, M. S. 1951. A quantitative study of Newcastle disease virus in tissues of infected chickens. Amer. J. Vet. Res. 12: 334-339.
4. Karzon, D. T., and F. B. Bang. 1951. Pathogenesis of infection with a virulent (CG 179) and a avirulent (BI) strain of NDV in chicken. I. Comparative rate of viral multiplication. J. Exp. Med. 93:267-284.
5. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent end points. Amer. J. Hyg. 27:493-497.
6. Rubin, H., and R. M. Franklin. 1957. On the mechanism of Newcastle disease virus neutralization by immune serum. Virology 8:84-95.
7. Rubin, H., R. M. Franklin, and M. Baluda. 1957. Infection and growth of Newcastle disease virus (NDV) in cultures of chick embryo lung epithelium. Virology 3:587-600.
8. Schloer, G. M., and R. P. Hanson. 1968. Relationship of plaque size and virulence for chickens of 14 representative Newcastle disease virus strains. J. Virol. 2:40-47.
9. Sinha, S. K., R. P. Hanson, and C. A. Brandly. 1952. Comparison of tropism of six strains of Newcastle disease virus in chickens following aerosol infection. J. Infec. Dis. 91: 276-282.
10. Singh, K. V., N. 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. 28:638-640.