DETECTION AND IDENTIFICATION OF THE NEWCASTLE DISEASE VIRUS INFECTION BY ELECTRON AND IMMUNOELECTRON MICROSCOPY

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Summary. – Various electron microscopic (EM) and immunoelectron microscopic (IEM) techniques were used to demonstrate and identify the Newcastle disease virus (NDV) infection. By IEM, the number of virions in native allantoic fluids was increased 50 – 100 times in comparison with direct EM. The immunogold staining showed that a number of immunogold particles were specifically bound to the antigen determinants located on the virion surface and these results were much easier to interpret. The obtained results showed that the EM and IEM can be successfully employed for a precise and rapid detection of NDV as well as for identification of this infection among other viral or bacterial infections.

Key words: Newcastle disease virus; detection; identification; electron microscopy; immunoelectron microscopy; immunogold method

Newcastle disease is a world-wide highly contagious avian disease caused by NDV and continues to be a serious economic threat to the poultry industry (Spradbrow, 1988; Alexander, 1991). An early and precise diagnosis is important for the prevention of the spread of the disease. A number of methods were developed for diagnosis of this infection, including pathological examination, isolation, identification and characterisation of the virus, as well as serological and biological tests. Occasionaly, an immunoperoxidase technique for thin sections (Hamid *et al.*, 1988; Wang Ching-Ho, 1992), immunoflourescence techniques for impression smears (McNulty *et al.*, 1986) and thin sections of trachea (Hilbink *et al.*, 1982), and EM examinations of gut content and allantoic fluids (Pospisil *et al.*, 1991) have been described and used as rapid methods for a direct detection of the virus.

The present study was undertaken to compare various EM and IEM techniques and to select the most appropriate one for a rapid detection and identification of NDV in allantoic fluids of infected chick embryos as a model system.

Abbreviations: BSA = bovine serum albumin; EM = electron microscopy(ic); HA = haemagglutination; HAI = haemagglutination-inhibition; IEM = immunoelectron microscopy(ic); NDV = Newcastle disease virus; PBS = phosphate-buffered saline

Viruses. La Sota, Glozene and Pavlikeni strains of NDV were grown in 10-day-old chick embryos (Hanson, 1980). The haemagglutination (HA) titers were assayed by a routine procedure.

Chicken anti-NDV sera. Sucrose gradient-purified La Sota strain (diluted 1:100) was administered intramuscularly to three chickens followed by three booster injections at 4-week intervals. Then the chickens were inoculated twice with Glozene strain and once with Pavlikeni strain at 4-week intervals. Two weeks after the last injection the animals were bled, the antisera were collected and tested for antibodies by haemagglutination-inhibition (HAI) test (Doumanova *et al.*, 1986).

Preparation of chicken anti-NDV-IgG-gold conjugate. Immunoglobulins from chicken anti-NDV sera were precipitated three times with saturated ammonium sulphate to 33% saturation and desalted on a Sephadex G 25 column (2.4 x 30 cm) equilibrated with

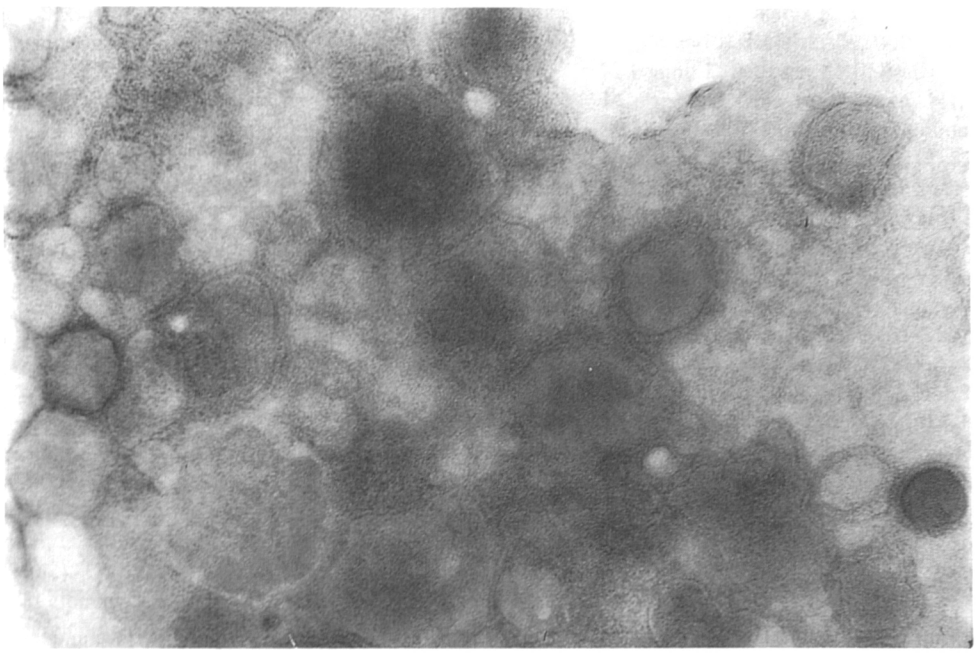

Fig. 1
EM of NDV particles concentrated by pelleting

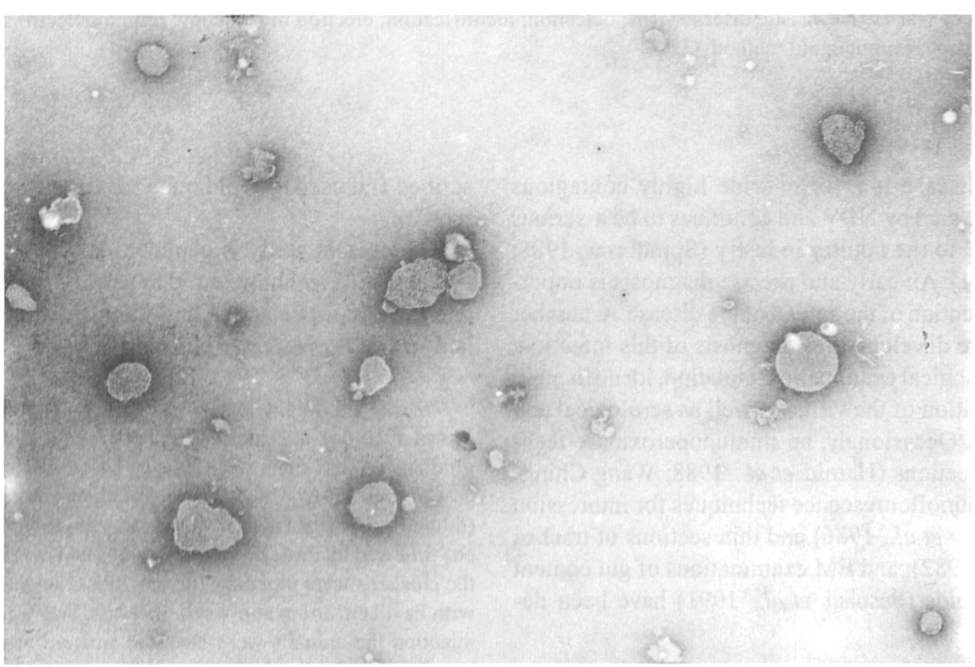

Fig. 2
IEM of NDV particles
Anti-NDV-IgG used.

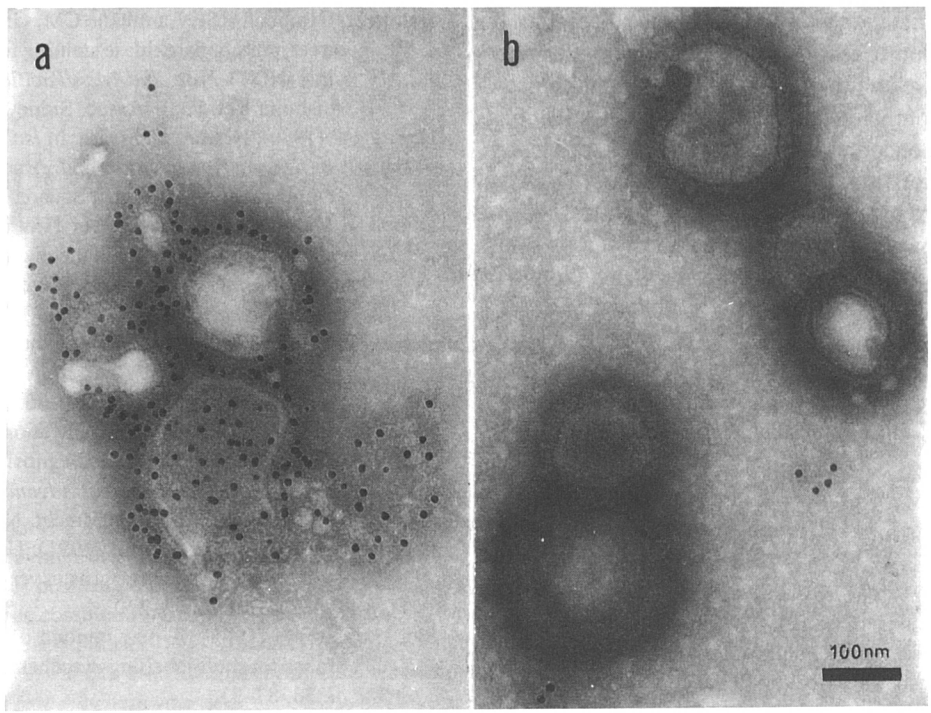

Fig. 3
IEM of NDV particles stained with gold
Conjugated (a) and non-conjugated anti-NDV-IgG (b) used.

0.01 mol/l phosphate buffer pH 7.2. Then they were rechromatographed on a column of DEAE-Sephadex A 50, equilibrated with the same buffer and the fraction corresponding immunoelectrophoretically to chicken IgG was eluted with 0.01 mol/l phosphate buffer pH 7.2 containing 0.05 mol/l NaCl. It was dialysed against 2 mmol/l borate buffer pH 9.0 and adsorbed to 10 nm colloidal gold, prepared according to Slot and Geuse (1985). The immunogold conjugate was washed three times by centrifugation at 60,000 x g for 1 hr at 4°C in a Beckman 55Ti rotor and the final pellet was resuspended in 0.01 mol/l Tris.HCl buffer pH 8.2, containing 0.85% NaCl, 0.1% NaN_3 and 1% bovine serum albumin (BSA), filtrated through 0.22 μm Millipore filter and stored at 4°C.

Samples. Allantoic fluids of 11-day-old chick embryos experimentally infected with different NDV isolates at different passage level were clarified by centrifugation at 2,000 x g for 30 mins at 4°C. The virus was pelleted by centrifugation at 109,000 x g for 1 hr at 4°C, redissolved and analysed by EM and IEM.

EM. Formvar- and carbon-coated 400-mesh copper grids were floated on a drop of the tested materials. The negative staining was performed with 2% sodium phosphotungstate pH 6.8 as described earlier (Horne, 1965; Alexandrov *et al.*, 1993). Samples were examined in a JEOL 1200 EX EM operating at an accelerating voltage of 80 kV and instrumental magnification of 20,000 – 50,000 x.

IEM. The grids were floated for 30 mins on a drop of chicken anti-NDV-IgG diluted 1:100, washed with PBS several times, floated on a drop of the examined materials for 1 hr and negatively stained as described above. Some of these preparations were additionally stained with the anti-NDV-IgG-gold conjugate and after negative staining they were examined by EM. A blocking test was used to control the specificity of the immunogold staining. In this test, before the immunogold staining, the grids were floated on a drop of non-conjugated chicken anti-NDV-IgG.

The EM examinations demonstrated single viral particles in native allantoic fluids, originating from chick embryos experimentally infected with NDV. The number of viral particles highly increased after the concentration of the NDV preparations by pelleting (Fig. 1).

Using IEM, the number of virions in native allantoic fluids increased 50 to 100 times in comparison with the direct EM examination (Fig. 2).

The immunogold staining demonstrated a number of gold particles specifically bound to the antigenic determinants located on the surface of virions (Fig. 3a). This staining gave good results that were much easier to interpret owing to the virtual absence of a non-specific staining in the blocking test (Fig. 3b).

The results presented in this communication warrant the conclusion that the EM and IEM techniques can be successfully employed for precise and rapid detection of Newcastle disease as well as to the identification of this infection among other viral or bacterial infections.

Using these methods we were able both to confirm the results of isolation trials and exclude any laboratory contaminations as well as to contribute to the right choice of optimal methodology of further laboratory investigations.

Based on our encouraging results, studies in our laboratory are in progress to identify an NDV infection in pathological specimens by use of EM and IEM methods, because many viral and bacterial agents occur almost always simultaneously and complicate the diagnosis (Wang Ching-Ho, 1992).

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References

Alexander DJ (1991): Newcastle disease and other paramyxovirus infections. In Calnek BW (Ed.): *Diseases of Poultry, Ames, Iowa.* Wolfe Publishing Ltd., pp. 496–519.

Alexandrov M, Peshev R, Bozkov S, Janchev I, Doumanova L (1993): Electron- and immunoelectron microscopic investigation on the rabbit haemorrhagic disease virus. *Comp. Immunol. Microbiol. Infect. Dis.* **16**, 21–27.

Doumanova L, Wassilewa L, Tsankova K (1986): Detection of ligands against M-protein on the surface of NDV-virions. *Vet. Sci.* **23**, 3–8.

Hamid H, Campbell RSF, Lamihane CM, Graydon R (1988): Indirect immunoperoxidase staining for Newcastle disease virus (NDV). *Proc. 2ndAvian/Pacific Poult. Health Conf.,* Australia Vet. Poult. Assoc. Sidney, pp. 425–427.

Hanson RP (1980): Newcastle disease. In *Isolation and Identification ofAvian Pathogens.* 2nd Ed.,American Assoc. ofAvian Pathologist, Texas, Kennett Square, PA, pp. 63–66.

Hilbink F, Vertommen M, Vant't Veer JTW (1982): The fluorescent antibody technique in the diagnosis of a number of pultry disease: Manufacture of conjugates and use. *Tijdschr. Diergeneessk.* **107**, 167–173.

Horne RW (1965): Negative staining methods. In Kay DK: *Techniques for Electron Microscopy.* Blackwell Scientific Publications, Oxford, pp. 328–355.

McNulty MS, Allan GM (1986): Application of immunofluorescence in veterinary viral diagnosis. In McNulty MS, McFerran JB (Eds): *Recent Advances in Virus Diagnosis.* Martinus Nijhoff, Dordrecht, Neth., pp. 15–26.

Pospisil Z, Zendulkova D, Smid B (1991): Unexpected emergency of Newcastle disease virus in very young chicks. *Acta Vet. (Brno)* **60**, 263–270.

Slot G, Geuze H (1985): A new method of preparation of gold probes for multilabelling cytochemistry. *Eur. J. Cell Biol.* **38**, 87–93.

Spradbrow PB (1988): Geographical distribution. In Alexander D (Eds): *Newcastle Disease.* Kluwer Acad. Pub., Norwell, pp. 247–255.

Wang Ching-Ho (1992): Application of immunoperoxidase staining for Newcastle disease. *J. Chin. Soc. Vet. Sci.* **18**, 87–92.