Properties of a Newly Isolated, Serologically Distinct Avian Paramyxovirus

By
D. J. Alexander¹, K. F. Shortridge², M. S. Collins¹, and N. J. Chettle¹

¹ Poultry Department, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England
² Department of Microbiology, University of Hong Kong, Pathology Building, Queen Mary Hospital Compound, Hong Kong

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Summary

The morphological, bio-physical and growth properties of the isolate duck/Hong Kong/D3/75 (D3/75) were consistent with this virus being a member of the paramyxovirus group.

Using haemagglutination inhibition and neuraminidase inhibition tests no serological relationships between D3/75 and other paramyxoviruses could be demonstrated.

The structural polypeptides of D3/75 were also typical of paramyxoviruses, consisting of 6—7 polypeptides ranging in molecular weight from 46,000—200,000 under reduced conditions. Two polypeptides were glycosylated.

Introduction

During the course of a surveillance programme for influenza in poultry in Hong Kong (17) many isolations of apparent paramyxoviruses were made from chickens, ducks and geese. Some of these were identified as Newcastle disease virus (NDV) (16) while others were shown to have no serological relationship with any of the known avian paramyxoviruses although forming a serologically homogeneous group (15). The object of the present paper is to describe the properties of a representative of these isolates, duck/Hong Kong/D3/75, and its relationship to other paramyxoviruses.

Materials and Methods

Viruses and Serological Tests

The viruses, sera and serological tests used in this study were as described (2, 15). The abbreviations used for the avian paramyxoviruses were: PMV/finch/N. Ireland/
Bangor/73 (Bangor), PMV/chicken/California/Yucaipa/56 (Yucaipa), PMV/parakeet/Netherlands/449/75 (449) and PMV/turkey/Wisconsin/68 (Ty/Wis). Duck/Hong Kong/D3/75 (D3/75) was chosen as a representative virus for the serologically related Hong Kong isolates. This virus was originally designated Duck/Hong Kong/D3/76 (15) but was in fact isolated in November 1975. The avian adenovirus strain CELO-Phelps was also used.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was based on the method of MAIZEL (10). Polyacrylamide gels 80 × 7 mm were prepared in glass tubes by polymerizing a solution containing: acrylamide 10 per cent, N,N-methylenebisacrylamide 0.27 per cent, N,N,N',N'-tetramethylethylenediamine 0.05 per cent, sodium dodecyl sulphate (SDS) 0.1 per cent in 0.375 M Tris-HCl buffer pH 8.9 with 0.06 per cent ammonium persulphate.

Purified virus samples were prepared with two per cent SDS, five per cent sucrose, 0.001 per cent bromophenol blue and, when reduced conditions were required, two per cent mercaptoethanol. Samples were heated for two minutes at 100 °C and 100 μg of protein in 0.1 ml layered onto each gel. The running buffer contained 28.8 g glycine and 6 g Tris per litre and was at pH 8.4. A constant 50 volts were applied for 3.5 to 4 hours by which time the bromophenol blue marker was about one cm from the end of each gel.

Polypeptides were detected by staining with 0.1 per cent Coomassie brilliant blue and glycopolypeptides by staining with Schiff's reagent as described (3).

The molecular weights of the polypeptides were estimated by comparison of the migration following SDS-PAGE with standards of known molecular weight. The standards used were: bovine serum albumin, human gammaglobulin and the polypeptides of the Ulster strain of NDV using the polypeptide molecular weights reported by Moore and Burke (12).

Cell Cultures

Chick kidney (CK) cells and chick embryo (CE) cells were prepared as described (4, 5).

Fusion experiments were done using Leighton tube coverslip cultures.

Percentage polykaryocytes, fusion indices and fusion events per cell were calculated as described (4, 8).

Virus growth experiments were done in 5 cm Petri dish cultures. To test its effect on virus growth 5-iodo 2-deoxyuridine (IDU) at a concentration of 25 μg/ml was added to the maintenance medium.

Haemadsorption

The method used for the measurement of haemadsorption to infected cells was based on that of FINTER (7). Monolayers of CK cells in five cm plastic Petri dishes were infected with approximately one EID50 per cell; at specified times after infection cultures were washed with ice-cold phosphate buffered saline pH 7.2 (PBS) and two ml 0.5 per cent chicken red blood cells (RBC) at 4 °C, were added and left for 30 minutes at 4 °C. The cell monolayer was then washed three times with ice-cold PBS and one ml 0.2 N sodium hydroxide added and left five minutes to dissolve the cells. The solubilized cells were added to one ml pyridine and the dish washed with one ml distilled water which was added to the pyridine. After thorough mixing the yellow colour of the solution was measured spectrophotometrically at 400 nm against an uninfected cell blank.

Results

Morphology

Negative contrast electron microscopy of infectious allantoic fluid showed D3/75 virus to have typical paramyxovirus morphology (Fig. 1). The pleomorphic particles had an average diameter of about 170 nm and a range of 100—300 nm,