AVIAN DISEASES vol. 20 no. 2

Detection of Four Serotypes of Avian Adenovirus in New Zealand

A. F. Green and J. K. Clarke
Department of Microbiology and Genetics
Massey University, Palmerston North, New Zealand

and

J. E. Lohr
Department of Veterinary Pathology and Public Health
Massey University, Palmerston North, New Zealand

Received 1 October 1975

SUMMARY

Twenty agents with adenovirus morphology were recovered from New Zealand domestic hens by means of chick kidney cell cultures. All the agents gave distinct cytopathic effects (CPE) in cell cultures, with two different types of CPE observed.

On the basis of neutralization tests, the twenty agents were assigned to four distinct serological groups. Physicochemical tests on a "prototype" strain from each serological group confirmed that these agents are adenoviruses.

The four prototype strains recovered in New Zealand are related to established overseas strains. One strain is serologically related to agents that cause IBH.

INTRODUCTION

Since the original isolation of an adenovirus (14) from a domestic fowl, it has become evident that these agents can be readily recovered from the domestic fowl population, and that they can be assigned to a number of serological types (2,7,9). It was recently demonstrated that at least two serological types of avian adenovirus are associated with inclusion body hepatitis (IBH) (3,10,13), which is an economically important disease in domestic fowls. That finding stimulated the present investigation to determine whether adenoviruses are present in the domestic fowl population of New Zealand, and whether serotypes (2,8) known to be responsible for IBH could be recovered.

The absence of reported outbreaks of IBH in New Zealand and the quarantine restrictions on importing viruses of vertebrates

236
into New Zealand led us to attempt isolation of IBH agents from the “normal” population with the object of classifying them serologically, and subsequently sending appropriate strains overseas for comparison with established strains.

**MATERIALS AND METHODS**

**Chick kidney cell cultures.** Fertile eggs were obtained from a flock of fowl maintained for research purposes at the Poultry Research Centre at Massey University, and incubated and hatched in isolation. Kidneys were removed aseptically from the chicks soon after hatching, dispersed by trypsin, centrifuged, washed twice with Earle's lactalbumin (LaE) solution, and the final pellet suspended in 400 times its volume in Hanks’ lactalbumin (LaH) medium containing 10% calf serum. Tubes, bottles, and petri dishes were seeded with this suspension, and before inoculation the medium was changed to LaE containing 2% fetal calf serum.

**Virus isolation and identification.** Specimens, mainly from the respiratory and alimentary tracts, were taken from domestic hens with a variety of clinical conditions, and from normal birds. Isolation of agents from the specimens was undertaken as described by McFerran et al. (9). This involved giving all specimens one “blind” passage in chick kidney cell culture, after which a specimen was regarded as negative if no cytopathic effect was observed.

When a CPE was observed, cells were scraped from the glass, collected by centrifugation, and lysed by the addition of a few drops of water followed by two cycles of freezing and thawing. The lysate was applied to carbon-coated grids, stained with 3% sodium phosphotungstate, pH 6.5, and examined by electron microscopy.

For the observation of inclusion bodies, cells were fixed with methanol or 10% formol saline, and stained with 1:75 dilution of Giemsa's stain for 24 hours.

**Physicochemical tests.** Chloroform sensitivity was determined by the method of Feldman and Wang (5), and the effect of cations on heat stability was determined by the method described by Wallis et al. (12).

The effect of 5-iodo-2-deoxyuridine (IUDR) on virus replication was investigated as described by McFerran et al. (9).

**Neutralization tests.** Antisera to “prototype” strains of viruses were prepared in rabbits. The agents used were passaged three times at limiting dilution in chick kidney cell cultures, and examined
Table 1. Cross-neutralization tests between prototype avian adenovirus strains.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Antiserum titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>5,120</td>
</tr>
<tr>
<td>B'</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>-</td>
</tr>
</tbody>
</table>

*Titer less than 40.

electronmicroscopically at final passage to minimize the possibility of having a mixture of viruses before they were used as immunizing antigens. The inoculation schedule and neutralization tests were as described by McFerran et al. (9).

RESULTS

Virus isolation and identification. During this investigation, 20 agents were recovered that were subsequently identified as adenoviruses. All gave an unequivocal CPE at least after one passage. However, not all adenovirus isolates gave an identical CPE: although most strains caused the formation of foci of rounded refractile cells, one strain (subsequently designated serotype D) consistently caused marked cell vacuolation as the first recognizable CPE, although the vacuolated cells eventually become rounded and refractile.

Intranuclear inclusions were observed in all stained preparations. These inclusions were similar to those described by Kawamura et al. (7) for avian adenoviruses.

All agents were observed by negative-contrast electron microscopy and were morphologically indistinguishable from each other and from human adenovirus type 5. All had a diameter of 70-85 nm, and triangular faces (Fig. 1, inset) were frequently observed on intact particles not penetrated by stain. Many particles were seen to be partially disrupted, but intact penetrated particles (Fig. 1) were typically hexagonal in outline. Individual capsomeres could be observed in some penetrated particles and were about 7 nm in diameter.

Physicochemical tests. Aliquots of the "prototype" strains of virus were titrated before and after exposure to chloroform. No significant change in the virus titer was detected.

Prototype strains were tested for heat stability (one hour at 50°C) in the presence of Na+, Mg++, and Ca++ ions. No significant
differences in virus titer was detected before and after heating in the presence of \( \text{Na}^+ \) or \( \text{Mg}^{++} \), but \( \text{Ca}^{++} \) destabilized the virus, giving rise in all cases to at least a 10,000-fold reduction in virus titer after heating.

Prototype strains of virus were inoculated into chick kidney cells in both the presence and absence of \( 10^{-4} \text{ M IUDR} \). Cultures were harvested and titrated as soon as the controls (without IUDR) showed an unequivocal CPE. With all four prototype strains, the yield of virus in the presence of IUDR was less than 1% of the titer of infectious virus in the control cultures; however, the inhibitory effect of IUDR was reversed by the addition of thymidine.

**Serological classification.** Following a preliminary investigation of serological relationships, four strains of avian adenoviruses, designated A, B, C, and D, were selected as "prototype" strains and used to prepare antisera. Cross-neutralization tests were then performed, using the four "prototype" strains and their antisera. The results are shown in Table 1.

Antiserum to the four "prototype" strains were tested for their ability to neutralize the remaining 16 isolates. Each isolate was neutralized by one and only one of the antisera. Thus, including the "prototype" isolates, one isolate was assigned to Group A, seven to group B, eleven to group C, and one to group D.

---

Fig. 1. Avian adenovirus negatively stained with 3% sodium phosphotungstate. Many particles are particularly disrupted. Triangular faces can be seen on two unpenetrated particles (inset). \( \times200,000 \).
DISCUSSION

All cytopathic agents recovered were indistinguishable in particle morphology from mammalian adenoviruses as described by Horne (6). Their resistance to chloroform indicates that they have no essential lipid, and the inhibition of their replication by IUDR gives presumptive evidence that they are DNA viruses. Furthermore, they were heat-stable in the presence of $2.0M \text{ Na}^+$ and $1.0M \text{ Mg}^{++}$ but were destabilized in the presence of $1.0M \text{ Ca}^{++}$, and it has been established that avian adenoviruses (7), like other adenoviruses (12), are destabilized in the presence of $1.0M \text{ Ca}^{++}$. We therefore conclude that all these agents are avian adenoviruses.

Twenty avian adenovirus isolates were divided into four distinct serological groups by neutralization tests, and no cross reactions were detected between the four “prototype” strains tested. Because of the difficulties involved in importing vertebrate viruses into New Zealand, the “prototype” strains were dispatched to the Veterinary Research Laboratories, Stormont, Belfast, where it was found (McFerran, personal communication) that in one-way typing tests using antisera to the standard avian adenovirus serotypes, each New Zealand “prototype” was neutralized by one, and only one, of the standard antisera: the New Zealand strain A is related to FA-4 (506), B to FA-1 (112), C to FA-8 (58), and D to FA-5 (340). The relationship of FA-1 through FA-8 to avian adenoviruses isolated by other workers has already been described (8).

McFerran et al. (8) recognize eight serotypes, and Calnek and Cowen (2) recognize ten serotypes of avian adenovirus. The present study found only four serotypes but was limited to flocks in the southern part of the North Island of New Zealand over a period of only 18 months. It is therefore possible that other serotypes of avian adenovirus are present in New Zealand but have not yet been isolated.

It is well known that adenoviruses can frequently be recovered from both diseased and apparently healthy birds (1,7,9,11), and this study is no exception: although most isolates were obtained from healthy birds, some strains were recovered from fowl with respiratory illness, and one isolate was recovered from birds which showed a sudden drop in egg production. It may be significant that one of the New Zealand serotypes (type D, identified as FA-5)
is serologically related to agents causing IBH. However, IBH has not yet been reported in New Zealand, so further experimental work is required to investigate the ability of our serotypes, especially strain D, to cause IBH. Such work is now in progress.

REFERENCES


ACKNOWLEDGMENTS

We acknowledge the support of the Poultry Research Center of Massey University, and the electron microscope unit of the D.S.I.R. (Palmerston North) during the course of this work.