**BRIEF NOTE**

**Avian Reovirus Isolated from Dead-in-Shell Embryos**

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Avian reovirus was first isolated from the chronic respiratory disease of chickens by Fahey and Crawley [4] in 1954, and thereafter, isolation of this virus from various diseases of chickens such as tenosynovitis [7], hydropericardium [1, 8] and cloacal pasting [3] has been reported. As yet, there is no report on the demonstration of avian reovirus in dead-in-shell embryos. Attempts were made at this laboratory to isolate the virus from the dead-in-shell embryos collected in a hatchery with the positive results. This report describes the characterization and identification of the isolated viruses as an avian reovirus.

Ninety four of the dead-in-shell embryos derived from five breeder flocks were obtained from one hatchery in the city of Kumamoto. Emulsions of 10 per cent whole embryos and livers extracted from dead eggs at 10 and 21 days of incubation, respectively, were used for virus isolation.

As a result, nine cytopathic agents (9.6%) were isolated from these materials with chicken kidney monolayer cell cultures (Table 1). The isolates were cytopathic, producing complete destruction of the cell monolayer by the second or third passage and in stained preparation typical cytoplasmic inclusion bodies were observed. These isolates exhibited essentially the same characteristics in cytopathic effect. Physicochemical and biological studies on the representative isolate (97/74) were carried out. The isolate contained RNA, was between 50 and 100 nm in size, and was not inactivated by ethyl ether, chloroform, sodium desoxycholate, trypsin and pH 3.0. The isolate was stable at 50°C for one hour in the presence of bivalent cation (Mg++). The isolate was completely inactivated by heating at 56°C for 40 minutes or 65°C for 15 minutes. The multiplication of the isolate began in chicken kidney cells 2 days after infection and reached the highest value at 7 days. No hemagglutination of chicken, mouse, guinea pig, rabbit, goat, sheep and cattle red blood cells was demonstrated at 4, 22 and 37°C. The isolate failed to produce illness in suckling and 3-week-old mice, guinea pigs and rabbits.

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**Table 1. Isolation of avian reoviruses from dead-in-shell embryos in chicken kidney cell cultures**

<table>
<thead>
<tr>
<th>Age of dead-in-shell embryos in days</th>
<th>Breeder flock</th>
<th>No. of tested samples</th>
<th>No. of strains isolated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>1 (20.0)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>1 (12.5)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>16</td>
<td>2 (12.5)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>38</td>
<td>4 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>9 (9.6)</td>
<td></td>
</tr>
</tbody>
</table>

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短報：ニワトリ死亡、胎児からのレオウイルスの分離：山田進二・上川慎一・内田洋一（化学及血清療法研究所）

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Immunoserum prepared against the 97/74 isolate neutralized the others as well as the homologous. Agar gel precipitin tests revealed antigenic relationship between the 97/74 isolate and the Uchida strain [5] of avian reovirus. The physicochemical, biological and immunological characteristics of the isolates suggest that they can be identified as an reovirus.

The possibility of egg transmission in avian reovirus infection has been suspected [2, 6, 9]. Deshmukh and Pomeroy [2] and Menendez et al. [9] reported the recovery of the virus from chicks derived from infected breeders. In their cases, it seems difficult to eliminate a possibility of contamination after hatching. Heide and Kalbac [9] observed the appearance of cytopathic effect in chick embryo fibroblast cultures prepared with 9-day-old embryos derived from breeders infected with the virus. When inoculated with chorioallantoic membranes infected with some of these cultures, chickens produced antibody against the virus, but they failed to detect specific antigenicity in the cultures with cytopathic effect.

The present work of virus demonstration in the dead-in-shell embryos provides direct evidence for egg transmission of avian reovirus.

References