BRIEF NOTE

Isolation of Newcastle Disease Virus from Imported Parrots (*Katakōe sulphurea*)

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During a recent study of viral diseases of psittacine birds, Newcastle Disease virus (NDV) was isolated from two parrots (*Katakōe sulphurea*) originating from the Republic of Indonesia. Parrots were imported as a single batch in May, 1979. Within a few days after quarantine, our laboratory was requested to investigate two diseased parrots from an importer in Aichi prefecture. The parrots were adults of unknown age. Two parrots showed slight nervous signs with tremor and died between 4 and 5 days after import. However, the other parrots or psittacine birds in the same flock never showed any symptoms of the disease. At necropsy, the parrots had hyperemia, petechial or ecchymotic hemorrhages, and regularly necrosis of the gastrointestinal mucosa.

The fertile eggs for embryo inoculation and chick embryo fibroblast (CEF) cultures were obtained from a specific pathogen free flock maintained by our laboratory. Samples were taken from the liver and spleen of dead birds and 10 percent organ emulsions (a mixture of liver and spleen) were inoculated into 11-day-old chicken embryo-

ating eggs as described previously [9]. Procedures for virus growth, cell culture, serology, hemagglutination and physio-

chemical examination were as described [7, 8]. Further characterizatoin of the isolates was carried out as suggested by Han-

son [5].

Hemagglutinating agents were isolated from the two parrots examined. Chick embryos died between 24 and 48 hours post-

 inoculation. The most constant lesions observed in the affected embryos were sub-

cutaneous hemorrhage. The virus titer of the chorioallantoic fluid was found to be $10^8.0 \text{ ELD}_{50}/0.2 \text{ ml}$, and mean death time of a minimum lethal dose was 58 hours. The isolates were cytopathic for CEF cultures. The isolates produced large ($\geq 1.5 \text{ mm}$) clear plaques.

Physicochemical properties of the representative isolate, GND-1 strain, are shown in Table 1. The addition of IUDR to the medium of CEF cultures at the time of in-

fection did not prevent the development of CPE. The result indicates that the nucleic
Table 1. Physicochemical properties of the isolate GND-1 strain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Titer of treated virus (TCID$_{50}$/mL)</th>
<th>Titer of untreated virus (TCID$_{50}$/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUDR (20 µg/mL)</td>
<td>$10^7.5$</td>
<td>$10^7.5$</td>
</tr>
<tr>
<td>10% Chloroform, 20°C, 30 minutes</td>
<td>$&lt;10^9$</td>
<td>$10^7.5$</td>
</tr>
<tr>
<td>pH 3.0, 4°C, 30 minutes</td>
<td>$&lt;10^9$</td>
<td>$10^7.0$</td>
</tr>
<tr>
<td>56°C, 5 minutes</td>
<td>$10^3.5$</td>
<td>$10^7.5$</td>
</tr>
<tr>
<td>15</td>
<td>$10^0.7$</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>$&lt;10^0$</td>
<td></td>
</tr>
<tr>
<td>Filtration 220 nm</td>
<td>$10^6.5$</td>
<td>$10^7.0$</td>
</tr>
<tr>
<td>100</td>
<td>$10^4.3$</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>$&lt;10^0$</td>
<td></td>
</tr>
</tbody>
</table>

* At pH 7.2. ** Not filtered.

Table 2. Cross hemagglutination-inhibition tests with GND-1 isolate and other avian Paramyxovirus strains

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>GND-1</th>
<th>Miyadera</th>
<th>B 1</th>
<th>Yucaipa</th>
<th>Bangor</th>
<th>Kunitachi</th>
</tr>
</thead>
<tbody>
<tr>
<td>GND-1</td>
<td>256</td>
<td>256</td>
<td>64</td>
<td>&lt;8</td>
<td>64</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Miyadera</td>
<td>128</td>
<td>256</td>
<td>128</td>
<td>&lt;8</td>
<td>64</td>
<td>&lt;8</td>
</tr>
<tr>
<td>B 1</td>
<td>256</td>
<td>128</td>
<td>256</td>
<td>&lt;8</td>
<td>64</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Yucaipa</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>8</td>
<td>256</td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Bangor</td>
<td>64</td>
<td>32</td>
<td>32</td>
<td>8</td>
<td>256</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Kunitachi</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>256</td>
</tr>
</tbody>
</table>

HI titers represent reciprocals of serum dilution.

acid type of the isolate is RNA. The isolate was found to be thermolabile and acid-labile, and was sensitive to chloroform treatment. The virus passed through the 100 nm filter, but it did not pass through the 50 nm filter. The negatively stained preparation showed that the particles with spikes had an average diameter of 120 nm, and that the particles were typical pleomorphic Paramyxovirus (Fig. 1). The hemagglutinin of the isolates was stable at 56°C for 15 minutes.

Various degree of cross hemagglutination inhibition reaction among avian paramyxoviruses were observed (Table 2). The GND-1 strain cross-reacted with NDV but not significantly with antigens prepared from Yucaipa, Bangor and Kunitachi strains.

When 1- and 3-week-old chickens were inoculated orally with $10^{7.0}$ EID$_{50}$ of the GND-1 strain for pathotyping purpose all died within 3 days. When one-year-old chickens were inoculated orally all birds showed diarrhea and edema of head, and died 3 to 6 days after inoculation. The chickens had hemorrhagic visceral lesions at necropsy. Histopathology showed hemorrhage and edema in the gastrointestinal tract, lung and trachea. The virus was recovered from the tissues of all chickens used in evaluating the isolate.

From the biological, physicochemical and morphological characteristics, the isolate seems to belong to the Paramyxovirus, among them it most closely resembled NDV. Concerning the pathogenicity in chickens, the isolate was probably a velogenic strain of NDV.

It is known that captive or cage birds, especially psittacines have been implicated
in the spread of virulent ND to domestic poultry flocks in a number of different countries, such as Great Britain [1], Austria [4], Republic of South Africa [3] and the United States of America [11]. Psittacine birds are highly susceptible to experimental infection with virulent NDV [2]. It is generally said that modern air transportation has greatly facilitated national and international movement of all species of birds, whether domestic poultry or cage birds [10]. The air shipment of birds in the incubative stage of infection was reported by Hanson [6]. Since birds are to continue to be transported in large number, corresponding quarantine measures and captive period involving strict procedures must be established by the importing countries, with special reference to in Japan. As for several pet bird species after quarantine, the administrators in Japan should guide importers and pet shops.

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Kunitachi and Bangor strains of Paramyxovirus, and Dr. H. Kawamura, of the National Institute of Animal Health, Poultry Disease Laboratory, Gifu, for supply of Yucaipa strain of Paramyxovirus.

References


要　約
輸入オウムからのニューカッスル病ウイルスの分離（短報）：平井克哉・山下照夫・沢　英之・高橋誠・鳥倉省吾（岐阜大学農学部家畜微生物学教室）、槇木利昭・井上　聡（岐阜大学農学部家畜病 理学教室）——インドネシア共和国から1群50羽輸入され、検疫の翌日某業者において死亡した2羽のオウム（Kakatoë sulphurea）からニューカッスル病ウイルスの強毒株が分離された。愛玩用鳥類については輸入検疫の検討ならびに輸入業者、小鳥店などの行政指導が必要であろう。

Explanation of Figure

Fig. 1. Electron micrograph of the GND-1 isolate stained with phosphotungstic acid. Typical pleomorphic form was seen, and projections were present in the outer coat of the particles. ×150,000. Bar=100 nm.