A Newcastle Disease Virus Isolated from Pullet Showing Leg-Weakness

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ABSTRACT. Leg-weakness was observed in a field flock of 82-day-old chickens, and a mesogenic Newcastle disease (ND) virus (TY-1 strain) was isolated from the brain of one affected chicken which revealed non-purulent encephalomyelitis characterized by necrosis of motor nerve cells in the brain and spinal cord. Clinical signs and lesions were reproduced in 6-month-old specific-pathogen-free chickens by an experimental infection with the TY-1 strain. These results indicate the existence of ND virus infection, which induces leg-weakness and obvious pathological changes in the central nervous system in the field.—KEY WORDS: encephalomyelitis, leg-weakness, Newcastle disease virus.

Chickens, in a 82-day-old flock, showed leg-weakness at 22 days after introduction into a farm. And 3% of the chickens were dead or sacrificed. In addition, drop of egg production was observed in other laying flocks on the farm. No obvious gross change was recognized in the affected pullets. However, encephalomyelitis characterized by motor nerve cell necrosis was microscopically observed in the brain and spinal cord (Fig. 1). These histopathological changes resembled those of avian encephalomyelitis (AE), and clinical signs were also similar to those of AE [5, 10].

In order to examine the causal agent, we then collected brain, trachea and rectum aseptically from six birds which showed leg-weakness. For each of the organs, a 1:5 homogenate was prepared with sterile phosphate buffered saline, and centrifuged at 2,000 rpm for 10 min at 4°C. Each of the resulting supernatants was inoculated into two chicken kidney (CK) cell cultures and three 10-day-old specific-pathogen-free (SPF) eggs. For the purpose of the isolation of AE virus, five 6-day-old SPF eggs were inoculated via the yolk sac with 0.1 ml of each test material from the brain.

Cytopathic effects were examined in CK cell cultures at 2 or 3 days after inoculation. Fluorescent antibody (FA) test and hemaggultination-inhibition (HI) test were used to identify the isolated agents. As a result of the tests, ND virus was isolated from the brain of one chicken and adenoviruses were isolated from the trachea and the rectum of all chickens. However, AE virus was not isolated.

The isolated ND virus (TY-1 strain) showed mesogenic properties by the characterizations [2] as shown in Table 1. The ability of plaque formation in chick embryo fibroblast cell cultures and the size of the plaque of the TY-1 strain were same as those of the Miyadera strain [7]. But the mean death time of the minimum lethal dose in 10-day-old eggs of the TY-1 strain was longer than that of the Miyadera strain, and intracerebral pathogenicity index for day-old chicks of the TY-1 strain was also lower than that of the Miyadera strain. Cross-HI test showed no antigenic difference among TY-1, B1 [1] and Ishii [9] strains (Table 2).

The TY-1 strain was experimentally inoculated into 4 SPF chickens of 6-month-old. One milliliter of allantoic fluid, which con-
Fig. 1. Encephalomyelitis of spinal cord of affected bird in the field. Motor nerve cells are undergoing degeneration, and mild gliosis is observed. Hematoxylin-eosin stain. ×300.

Table 1. Biological characteristics of TY-1 strain

<table>
<thead>
<tr>
<th>Test system</th>
<th>TY-1</th>
<th>B1</th>
<th>Ishii</th>
<th>Miyadera</th>
<th>Sato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque formation in chicken embryo fibroblasts</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Size (mm)</td>
<td>ø2.5-3.0</td>
<td></td>
<td>ø2.5-3.0</td>
<td>ø1.5</td>
<td></td>
</tr>
<tr>
<td>Thermostability of hemagglutinin</td>
<td>medium</td>
<td>weak</td>
<td>medium</td>
<td>strong</td>
<td>medium</td>
</tr>
<tr>
<td>Rate of elution</td>
<td>slow</td>
<td>rapid</td>
<td>slow</td>
<td>intermediate</td>
<td>rapid</td>
</tr>
<tr>
<td>Mean death time of the minimum lethal dose (MDT/MLD)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.18</td>
<td>NT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NT</td>
<td>57.39</td>
<td>NT</td>
</tr>
<tr>
<td>Intracerebral pathogenicity index (ICPI)&lt;sup&gt;b&lt;/sup&gt; for day-old chicks</td>
<td>1.65</td>
<td>0.47</td>
<td>NT</td>
<td>1.83</td>
<td>NT</td>
</tr>
</tbody>
</table>

<sup>a</sup> (No. dead at X hour) × (X hour) + (No. dead at Y hour) × (Y hour) etc

<sup>b</sup> Total number of dead

<sup>c</sup> 0: Normal, 1: Diseased, 2: Dead. The resulting sum is divided by the number of observations.

<sup>d</sup> Not tested.

tained $1.5 \times 10^9$ plaque-forming units of virus per ml was inoculated intratracheally to each chicken. These chickens showed no clinical signs until 7 days after inoculation. At 8 days after inoculation, however, two chickens showed leg-weakness and 4 chickens were sacrificed for pathological examination. There was no obvious macroscopic change,
Table 2. Cross-hemagglutination inhibition test among three strains of Newcastle disease virus

<table>
<thead>
<tr>
<th>Antiserum against</th>
<th>Antigen</th>
<th>TY-1</th>
<th>B1</th>
<th>Ishii</th>
</tr>
</thead>
<tbody>
<tr>
<td>TY-1</td>
<td>128</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>64</td>
<td>128</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Ishii</td>
<td>32</td>
<td>64</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

a) Prepared in 3-month-old specific-pathogen-free chickens immunized with inactivated purified viruses.

but by the histopathological examination, encephalomyelitis characterized by necrosis of motor nerve cells was observed in the brain and spinal cord in all chickens inoculated with the virus.

Generally, AE virus infects chickens of all ages, and it rarely induces clinical signs in adult chickens except for drop in egg production of laying hens [3, 4, 10, 11]. However, it has been reported in Japan that AE virus infection induced leg-weakness in adult chickens [5]. ND vaccines had been administered on the farm where this disease occurred. Therefore, AE virus infection was first suspected in this case.

On the other hand, it has been reported that the pneumonecephalitis type ND virus also induces similar clinical signs and pathological changes to those of AE [6].

In the present case, AE virus was not isolated, but ND virus inducing leg-weakness and encephalomyelitis experimentally was isolated. These results indicate the existence of ND virus in the field which induces leg-weakness and obvious pathological changes in the brain and spinal cord.

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REFERENCES