Recovery of Virus from Feces and Tissues of Chickens Infected with Cell-Culture-Adapted Infectious Bursal Disease Virus

Kozo TAKASE, Fujio NONAKA, Terutoshi FUKUDA and Shinji YAMADA

The Chemo-Sero-Therapeutic Research Institute 668 Ohkubo, Shimizu-cho, Kumamoto-shi, Kumamoto 860

(Received for publication January 16, 1981)

Abstract. Recovery of cell-culture-adapted infectious bursal disease virus (FK-78 strain) from feces and tissues of chickens infected experimentally with the virus was investigated. The virus was recovered from the feces, contents of caecum and jejunum, bursa of Fabricius, liver, spleen, thymus, kidney, and lung of chickens inoculated orally with the virus at 21 days of age. Virus titer was the highest in the bursa of Fabricius, and moderately high in the feces and the caecal contents. High virus titers were obtained 4 or 5 days after inoculation. On the other hand, the virus titers in the feces were influenced by the route of virus inoculation. High titers were obtained from chickens having received the virus orally or intranasally. The younger an inoculated chicken, the earlier the initial recovery of the virus from the feces of the chicken.

Infectious bursal disease virus (IBDV) infects susceptible young chickens, and replicates in the bursa of Fabricius (BF) and the other lymphoid organs. It is excreted with feces through the cloaca.

The transmission of IBDV in susceptible chicken flocks in the field has been considered to be rapid from the results of clinical and serological investigations [2, 4], and confirmed experimentally [1, 6]. Factors involved in the transmission [1] and the distribution [5, 7] of IBDV in chickens have been described. However, a virus excreted in feces and considered to be a causative agent transmitted has not been investigated experimentally at all. In this study, an attempt was made to recover IBDV quantitatively from feces and tissues of chickens infected with cell-culture-adapted IBDV and to investigate the titer and excretion period of the virus in feces.

Materials and Methods

Chickens and embryos: All the chickens used in this study were specific pathogen free (SPF) White Leghorns derived from the SPF parent flocks maintained at the authors' laboratory. Three of the 56-day-old chickens had agar-gel-precipitating antibody against IBDV. All the chickens were reared in isolation facilities during the experimental period. Embryos used for preparing chick embryo fibroblast (CEF) cultures were derived from the same SPF parent flocks.

Virus: Strain FK-78 was used as inoculum. It was a cell-adapted IBDV that had passed five times in embryonating chicken eggs and 48 times in CEF cultures after isolation from field materials. It showed cytopathic effect (CPE) on CEFs.

Cell culture: Primary CEF cultures were prepared from 10-day-old chick embryos. The embryos were decapitated, enervated, minced and trypsinized (0.25% trypsin) at room temperature for 15 minutes. The dispersed cell suspension was filtered through a wire net (200-mesh). After addition of 1% bovine serum (BS), it was centrifuged at 1,000 rpm for 5 minutes. The cell pellet was resuspended at a ratio of 10⁴ cells/ml in growth medium consisting of Eagle’s MEM (Nissui) with 10% tryptose broth (Difco), 0.03%, L-glutamine, 5%, BS and antibiotics (penicillin, 200 IU/ml, and streptomycin, 200 µg/ml). The pH of the growth medium was adjusted to 6.8 with 7% NaHCO₃ solution. The cell suspension was placed in test tubes (1 ml/tube)
and incubated at 37°C for 24 hours. Maintenance medium consisted of the same components as the growth medium, except that BS was reduced to 2%.

Virus assay: The tissues and feces collected were homogenized with phosphate-buffered saline (PBS) containing antibiotics (penicillin and kanamycin, 500 IU/ml, and streptomycin, 500 μg/ml) and made to 10% suspensions. After centrifugation of the suspensions at 3,000 rpm for 15 minutes, the supernates were used for virus assay. Tenfold serial dilutions of the supernates were made in PBS and 0.1 ml of each dilution was inoculated into each of 4 CEF-cultured tubes. Virus adsorption was done at 37°C for an hour. Then the tubes were washed once with PBS. To each tube was added 0.9 ml of maintenance medium for further incubation at 37°C. The appearance of specific CPE 4 days postinoculation (PI) was recorded. The titer (TCID<sub>50</sub>) was calculated by the method of Behrens-Kärber.

Experimental design.

Expt. I: Each of 42 SPF chickens 21 days old was inoculated with the FK-78 strain (10<sup>4.0 TCID<sub>50</sub>/bird) by the oral route. On days 0 to 12 (daily) and 17 PI, 3 birds each were sacrificed to collect samples for virus assay. The samples were derived from the contents of caecum and jejunum, and tissues from liver, spleen, kidney, heart, lung, thymus and BF. They were stored at −70°C until use for assay.

Expt. II: Twenty SPF chickens 21 days old were divided into 4 groups and administered with the FK-78 strain (10<sup>4.0 TCID<sub>50</sub>/bird) by the oral, intranasal, intraocular and intramuscular routes, respectively. Five birds of each group were transferred to another cage for 2 hours daily for 14 days after inoculation and allowed to pick up feces excreted. Fecal samples were collected daily and pooled in each group. They were stored at −70°C until use for assay.

Expt. III: Three each of SPF chickens 2, 21, 56 and 105 days old and three 56-day-old chickens which had antibody against IBDV were inoculated with the FK-78 strain (10<sup>4.0 TCID<sub>50</sub>/bird). Feces were collected daily from them. Feces collected from 2-day-old chickens were pooled daily, while those from the other chickens were collected individually in the same manner as described in Expt. II. These feces were stored at −70°C until use for assay.

Results

The results of Expt. I are shown in Table 1 and Fig. 1. Table 1 presents the number of chickens in which virus was recovered from tissues. The virus was detectable in many tissues but not in the heart. The virus was detected from the BF and the contents of the caecum one to 8 days PI in most of the chickens. It was recovered from various kinds of tissues examined 4 days PI. Virus titers in these tissues are indicated in Fig. 1 as the averages of 5 chickens. The titer

<table>
<thead>
<tr>
<th>Tissue*</th>
<th>Time in days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Caecum [contents]</td>
<td>**</td>
</tr>
<tr>
<td>Jejunum [contents]</td>
<td>*</td>
</tr>
<tr>
<td>Bursa</td>
<td>*</td>
</tr>
<tr>
<td>Liver</td>
<td>*</td>
</tr>
<tr>
<td>Spleen</td>
<td>*</td>
</tr>
<tr>
<td>Thymus</td>
<td>*</td>
</tr>
<tr>
<td>Kidney</td>
<td>*</td>
</tr>
<tr>
<td>Lung</td>
<td>*</td>
</tr>
<tr>
<td>Heart</td>
<td>*</td>
</tr>
</tbody>
</table>

Chickens were inoculated with 10<sup>4.0 TCID<sub>50</sub>/bird of the FK-78 strain. Three of them were sacrificed every day after inoculation to collect tissue samples.

* Ten percent homogenate.

** The dot indicates zero.

*** No. of chickens positive for virus recovery.
Fig. 1. Virus titers in tissues of chickens inoculated orally with infectious bursal disease virus.

Chickens were inoculated with $10^{5.0}$ TCID$_{50}$/bird of the FK-78 strain. Tissue samples were collected from 3 birds sacrificed every day. The virus titer is indicated as the average.

Coecal contents: □, bursa of Fabricius: ▲, liver: ○, spleen: ●, and thymus: △

Fig. 2. Virus titers in feces of chickens inoculated with infectious bursal disease virus by different routes.

Four groups of 5 chickens each were inoculated with $10^{4.5}$ TCID$_{50}$/bird of the FK-78 strain by intranasal (×—×), intraocular (○—○), intramuscular (□—□), and oral (▲—▲) routes, respectively. Feces from the 5 birds of one group were pooled every day for virus titration.

was the highest in the BF, and its peak ($10^{5.3}$ TCID$_{50}$/0.1 g) obtained 5 days PI. A moderately high titer was obtained in the contents of the caecum, and its peak ($10^{5.6}$ TCID$_{50}$/0.1 g) was seen 4 days PI. In most of the other tissues examined, peaks of virus titers were also seen 4 days PI.

The results of Expt. II are given in Fig. 2. Virus titers in the feces varied with the route of virus inoculation. They were rather high in the groups inoculated orally or intranasally with the virus. In the group inoculated intraocularly with the virus, the virus, although low in titer, was recovered for a long period up to 10 days PI. In the group inoculated intramuscularly with the virus, the virus was recovered only 5 days PI.

The results of Expt. III are shown in Fig. 3. When the virus was inoculated at 2 or 21 days of age, the virus in the feces was detected initially 1 or 2 days PI. On the other hand, when the virus was inoculated at 56 or 105 days of age, it began to be recovered 4 or 5 days PI. Moreover, there was a bird which excreted feces containing no detectable virus. No virus was recovered from the feces of chickens which had had antibody against IBDV prior to inoculation.
Discussion

The distribution of IBDV in susceptible chickens was reported by Winterfield et al. [7], Ide [3], and Skeeles and Lukert [5]. Winterfield et al. [7] recovered the virus from many tissues of chickens 4 to 5 weeks old inoculated with a virulent or vaccinal strain of IBDV. Skeeles and Lukert [5] investigated the location and titer of the virus in tissues of one-day-old chicks given cell-culture-adapted virus orally or subcutaneously. Ide [3] reported the isolation periods of the virus from tissues of 3-week-old chickens inoculated with the SK-1 strain by the intravenous or conjunctival route.

In the present study, the virus distribution and persistence in the BF were essentially the same as those found by the workers cited above [3, 5, 7], although the virus titer and isolation period in any other tissue than those mentioned above were low and short. Especially, the virus titer was low in the kidney and lung in the present study. It should be noted that the virus was recovered from the contents of the caecum as early as from the BF, and that the virus titer was substantially high in these contents. On the other hand, the virus was scarcely recovered from the contents of the jejunum.

Virus titer in the feces was influenced by the route of virus inoculation. It was high in chickens inoculated by the oral or intranasal route and low in chickens inoculated by the intraocular or intramuscular route. These results suggest that the virus replication rates in the BF and other tissues of chickens may vary with the route of virus inoculation. Skeeles and Lukert [5] pointed out that no virus was detectable in any tissue of birds injected subcutaneously with cell-culture-adapted IBDV at 5 weeks of age. In the present study, little virus was recovered from feces when injected by the intramuscular route.

It is interesting to note that the beginning time of virus recovery from feces was influenced by the age of chickens used. In birds inoculated with the virus at 2 or 21 days of age, the virus began to be detected from feces 1 or 2 days PI. It began to be recovered 4 or 5 days PI when inoculated at 56 or 105 days of age. The reason for this difference is unknown. It may be attributed, however, to the degree of development of the organs, especially the digestive organs, of the chickens used. It may require a rather long time for the virus to reach a target site in older chickens which have the well-developed digestive organs and effectively functional defence systems, as compared with younger chickens.

When the virus titer was high in the caecal contents and BF, it was also high in the feces. Then it is considered that the virus in the feces may perhaps be derived from these tissues.

References

要約

伝染性ファプリキウス瘧病ウイルス細胞髄化株接種親の糞および臓器からのウイルス回収  藤田公美・倉田正男・福田輝俊・山田俊二（化学及血清療法研究所）—伝染性ファプリキウス瘧病ウイルスの培養細胞髄化株（FK-78株）をSPF雛に接種し、糞および主要臓器からのウイルス回収を試みた。ウイルスは腎、盲腸、小腸、肝臓、脾臓、腎臓、肺臓、胸腺およびファプリキウス糞から回収された。ウイルス量はファプリキウス糞で最も多く、盲腸内および糞がこれに次ぎ、また各臓器におけるウイルス量は接種後4〜5日目にピークに達した。糞から回収されるウイルス量は、ウイルスの接種ルートにより異なり、経口あるいは点鼻接種糞では比較的多量に回収され、点眼接種糞では減少し、筋肉接種糞ではきわめて少なかった。ウイルスを2あるいは21日齢に経口接種した雄の糞からは、接種後1〜2日目より10日目まで回収されたが、接種日齢が56あるいは105日齢と高くなると、ウイルスの回収時期は遅れ、接種後4〜5日目から回収され始め、しかも回収期間も短くなる傾向にあった。