Comparative Studies of Physicochemical and Biological Properties between Canine Parvovirus and Feline Panleukopenia Virus

Hitoshi GOTO, Toshihiro HIRANO, Eiji UCHIDA¹, Kiyoka WATANABE¹, Morikazu SHINAGAWA², Shigeru ICHIJO¹, and KiheiJI SHIMIZU

Department of Veterinary Microbiology, ¹Department of Veterinary Medicine, and ²Department of Veterinary Public Health, Obihiro University of Agriculture and Veterinary Medicine, Inadaecho, Obihiro, Hokkaido 080, Japan

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ABSTRACT. The properties of canine parvovirus (CPV) and feline panleukopenia virus (FPLV) were compared by means of physicochemical treatments, biological tests, and experimental infection of domestic dogs and cats or specific pathogen-free (SPF) cats. No distinct difference between CPV and FPLV was observed in their physicochemical properties. The difference between CPV and FPLV in range of host cells and in hemagglutination could be confirmed in this study. Moreover, CPV was inactivated by heating at 80°C for one hour as compared to the inactivation of FPLV at 80°C for 2 hours. The physical map of CPV differed from that of FPLV at both ends of DNA digested with Bgl II and Hind III, though the 2 fragments in central part were common to the viruses. Experimental infection of domestic dogs with CPV or FPLV and domestic cats with CPV produced clinical signs of the disease, while the CPV-inoculated SPF cats did not show any symptoms. However, all the experimental animals, with or without clinical signs, produced hemagglutination-inhibiting antibodies to the virus.—Key words: canine parvovirus, comparative properties, feline parvovirus.


INTRODUCTION

Canine parvovirus (CPV) infection is a fatal disease of young dogs with vomiting, anorexia, diarrhea and rapid dehydration. In 1978, many outbreaks of the disease were recognized almost simultaneously throughout the world [4]. Since then, a number of investigations of serology, virology, and clinical aspects of the disease have been reported from many countries, including Japan [3, 20]. The clinical signs of the disease closely resembled feline panleukopenia virus (FPLV) infection in cats. An antigenic similarity between CPV and FPLV using various serological methods was described by many investigators [1, 2, 3, 5, 7, 11, 12, 13, 15]. While some differences between the viruses were demonstrated by agar gel precipitin tests [6, 16] and analysis of viral DNAs [19]. This paper concerns the comparative studies of physicochemical and biological properties between CPV and FPLV, and an experimental infection of dogs and cats with the viruses.

MATERIALS AND METHODS

Cells and viruses: FLF-3 cells, a feline lung cell line, were used at high passage level (>80 in this laboratory) as described previously [9]. The cells were grown in Dulbeco's modified Eagle's medium supplemented with 4% fetal calf serum and antibiotics. MDCK (canine kidney), MDBK (bovine kidney), MA-104 (rhesus monkey kindey) and HaLa (human cancer) cells cultured in Eagle's minimum essential medium containing 8 to 10% calf serum and antibiotics were used for the investigation of host cell range of the virus. All the cell lines were supplied by the Department of Hygiene and Epidemiology, Sapporo
Medical College, Sapporo.

CPV (strain Kushiro) was isolated in 1982 from the intestine of a diseased dog. The dog was 3-month-old male raised in Kushiro, Hokkaido, and the animal showed severe diarrhea with some blood and vomiting. It died 3 days after the onset of first clinical signs. The virus was used at the 7th to 9th passage levels in FLF-3 cells, having a titer of $10^6$ TCID$_{50}$/ml [Goto, et al., *The 95th Meeting of Jpn. Soc. Vet. Sci.*, 1983]. FPLV (strain Obihiro) was isolated [10] and serially passed 8 to 12 times in secondary feline kidney (FK) cells and 2 to 8 additional passages in FLF-3 cells, having a titer of $10^7$ TCID$_{50}$/ml. Virus isolation and titration of both CPV and FPLV were carried out according to the methods fully described elsewhere [9].

**Physicochemical treatments:** The sensitivity of the viruses to 20% ethelether, 5% chloroform, low (3.0) and high (8.0) pH, heating at 56°C for 30 minutes and 80°C for 10 to 120 minutes, and the filtrability of the virus through Millipore filters of 100, 50 and 25 nm were examined using previously described methods [10].

**Electron microscopy (EM):** Intestine specimens were collected from the affected dog mentioned above with CPV, and from the specific pathogen-free (SPF) cats sacrificed at 4 and 5 days postinoculation (DPI) with FPLV in our previous experiment [9]. About 5 ml of a 10% (W/V) suspension of the intestine specimens in Eagle’s minimum essential medium containing 2% calf serum and antibiotics was centrifuged at 8,500xg for 30 minutes. The supernatant fluid (3 ml) was layered onto 2 ml of 25% sucrose in 10 mM phosphate-buffered saline, pH 7.5 (PBS) and centrifuged at 100,000xg for 3 hours (Hitachi 65P). The resulting pellet was suspended in 3.5 ml of PBS, and cesium chloride was added to the suspension to a final density of 1.39 g/cm$^3$. Centrifugation was carried out for 17 hours at 100,000xg. Fractions of 0.2 ml each with densities between 1.38 to 1.40 g/cm$^3$ were collected and suspended in 4.5 ml of PBS. Recentrifugation was carried out for 3 hours at 100,000xg and the final pellet was suspended in 0.05 ml of double distilled water. The virus suspension was mixed with an equal volume of 3% uranyl acetate (pH 4.0) and used for EM observation as described previously [18].

**Hemagglutination (HA) and hemagglutination-inhibition (HI) tests:** The HA and HI tests were performed by essentially the same method as described elsewhere [8], except the use of diluent consisting of 7 mM PBS, pH 6.8 supplemented with 0.04% bovine albumin and 0.03% gelatin.

**Experimental animals:** The animals used for experimental infection with the viruses were 10 domestic dogs aged 1 to 3 months, 5 SPF cats 4 months of age, and 4 domestic cats 3 months old, of both sexes. The SPF cats were kindly supplied from the same colony, and the infected animals were maintained and sacrificed under the same manner, respectively, as described previously [9]. The domestic dogs and cats infected with the viruses were housed in individual cages and watched for clinical signs of disease every day from 5 day before to 11 days after the infection.

**DNA preparation and enzyme digestion:** Preparation of viral DNA has been described previously [17]. Restriction endonucleases *Bgl II* and *Hind III* used in this study were obtained from Takara-Shuzo (Kyoto).

**RESULTS**

**Physicochemical properties:** Experiments were performed using CPV at the 8th passage level in FLF-3 cells and FPLV at the 5th passage level in FLF-3 cells following 12 passages in FK cells. CPV and FPLV were completely resistant to the treatments of 20% ethelether at 4°C for 18 hours, 5% chloroform at 4°C for 20 minutes, and pH 3.0 or 8.0 at 20°C for 4 hours, respectively. Both viruses

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Table 1. Heat stability of CPV and FPLV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log_{10} titer in TCID_{50}/ml</th>
<th>CPV</th>
<th>FPLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>56°C 30 min</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>80°C 10 min</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>80°C 30 min</td>
<td>1.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>80°C 60 min</td>
<td>&lt;1.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>80°C 90 min</td>
<td>&lt;1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>80°C 120 min</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td></td>
</tr>
</tbody>
</table>

showed no decrease of infectivity after filtration with Millipore filters of 100, 50 and 25 nm in pore size.

As shown in Table 1, the viruses adjusted to the same titer of 10^5 TCID_{50}/ml were resistant against heating at 56°C for 30 minutes. For inactivation by heat at 80°C, however, CPV was inactivated partially after 10 to 30 minutes and completely within 60 minutes, while FPLV showed a gradual decrease of infectivity titers throughout the inactivation period from 10 to 120 minutes.

Virus particles: A great number of spherical particles were seen in negatively stained preparations from the CPV-infected dog (Fig. 1) and the experimentally FPLV-infected SPF cats (Fig. 2). The size of both virions was about 20 nm in diameter. No other kind of virus particles was recognized in the preparations.

Host cell range: The propagating ability of CPV and FPLV was studied in FLF-3, MDCK, MDBK, MA-104 and HeLa cells. Typical intranuclear inclusions were observed following inoculation with CPV at high dilutions up to 10^{-6} in FLF-3 cells (Fig. 3) and at low dilutions up to 10^{-3} in MDCK cells (Fig. 4). FPLV replicated only in FLF-3 cells inoculated with high dilutions up to 10^{-7}. No distinct difference with regard to the morphological aspect of intranuclear inclusions was observed between the 2 viruses. Neither virus propagated in the other cell lines used.

Hemagglutination: In an experiment with erythrocytes of several animal species (Table 2), CPV and FPLV agglutinated the erythrocytes from pig and Japanese monkey (Macaca fascata), while only CPV agglutinated feline erythrocytes. On the other hand, the viruses did not agglutinate the erythrocytes of cattle and guinea pigs. The HA titer of CPV was consistently at least 8 times higher than that of FPLV.

Experimental infection: All the experimental animals, 8 domestic dogs, 5 SPF cats and 4 domestic cats, showing no HI antibody to CPV were inoculated subcutaneously with 1 ml of CPV, at the 8th passage level in FLF-3 cells. The animals were sacrificed 3 to 11 DPI. Three of the 8 examined dogs showed clinical signs such as depression, anorexia, fever, vomiting, diarrhea and dehydration at 3 to 5 DPI, and one of them died at the 5th day. Marked decrease of leukocyte count was observed in all the cases. The remaining 5 dogs showed only slight decrease of leukocyte count. The inoculated 5 SPF cats showed neither clinical signs nor leukopenia through the observation period. In the 4 domestic cats, however, depression (4 cases), vomiting (2 cases), diarrhea (1 case) and severe leukopenia (4 cases) were found at 3 to 7 DPI. One of them died on the 4th day.

All the inoculated animals, irrespective of species and with or without clinical signs, had HI titers of 16 to 64 against CPV at 3 to 5 DPI. Four animals showed the HI titers of 128 to 512 at 7 DPI, and the titers elevated to as high as 2,048 or over at 11 DPI in 2 cases. When 9 animals were sacrificed at 3 to 5 DPI, CPV was detected in such as liver, lung, spleen, kidney, bone marrow, thymus, mesenteric lymph nodes and intestines in all the cases. The virus could not be isolated from any of the organs of the sacrificed animals at 7 to 11 DPI.

Two domestic dogs, HI antibody negative to FPLV, were inoculated subcutaneously with 1 ml of FPLV, 8 passages in FK cells and 3 additional passages in FLF-3 cells.
Figs. 1 and 2. Negatively stained virus particles from the intestine specimens of the dog infected naturally with CPV (Fig. 1) and the SPF cats infected experimentally with FPLV (Fig. 2) ×150,000 (Bar=100 nm).

Figs. 3 and 4. Intranuclear inclusions of CPV in FLF-3 cells (Fig. 3) and in MDCK cells (Fig. 4). May-Grünwald-Giemsa stain. ×400.
Table 2. Hemagglutination of CPV and FPLV

<table>
<thead>
<tr>
<th>Erythrocyte</th>
<th>HA titer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CPV</th>
<th>FPLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>5,120</td>
<td>640</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>5,120</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>640</td>
<td>&lt;4</td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The titer was expressed as the reciprocal of the highest dilution of virus which had completely agglutinated erythrocytes.

**[a] Enzyme digesion**

<table>
<thead>
<tr>
<th>None</th>
<th>Bgl II</th>
<th>Hind III</th>
<th>Bgl II + Hind III</th>
<th>M. W. marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>C</td>
<td>F</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Origin</td>
</tr>
</tbody>
</table>

Fig. 5. Gel electrophoresis patterns of restricted DNAs [a] and comparative restriction maps of DNAs [b] of CPV (C) and FPLV (F). Molecular weight marker: PBR 322, Hae II, Hind III and Hind II fragment mixture.
The dogs were observed for clinical signs every day from 5 days before to 11 days after the inoculation. Both dogs showed slight diarrhea and slight decrease of leukocyte count at 4 to 8 DPI and one dog had vomiting at the 4th day. Virus showing intranuclear inclusions typical of FPLV in FLF-3 cells was detected from fecal specimens at the 6th day in both cases. The dogs had HI titers of 64 to 256 to FPLV at the 3rd day and 256 to 2,048 at 7 to 11 DPI.

Restriction enzyme analysis: The double-stranded replicating form DNA of CPV and FPLV was prepared from the infected FLF-3 cells. Fig 5 [a] shows agarose gel electrophoresis patterns of restricted DNAs of both viruses. The undigested DNA of FPLV and CPV migrated almost similar. Digestion with Bgl II generated 2 fragments in each virus, though there were no common DNA bands in the migration picture. Three fragments of viral DNAs were produced by Hind III, and both viruses shared the smallest of them. Double digestion with Bgl II and Hind III revealed 4 fragments as expected in the viruses (the DNA band of the largest fragment of CPV is doublet). The 2 small fragments were shared by both FPLV and CPV. Molecular weights of FPLV and CPV DNAs, estimated according to the sum of fragment sizes compared with molecular weight markers, are $3.2 \times 10^6$ and $3.4 \times 10^6$, respectively. FPLV-DNA was 6% smaller than CPV-DNA. In the comparative physical maps of FPLV and CPV, as shown in Fig. 5 [b], 2 fragments of 660 and 900 base pairs located in central part were common to the 2 viruses, while there was considerable difference at both end fragments.

DISCUSSION

In the present study, no significant difference between CPV and FPLV was recognized in their resistance to organic solvents, strong acid or alkaline pH, and in the shape and size of virus particles. As a useful criterion for distinguishing CPV from FPLV, their hemagglutination behaviour and their host range in cell cultures have been mentioned by several workers [2, 5, 7, 11, 12, 15]. These criteria were confirmed by the results obtained in the present study. That is, CPV and FPLV agglutinated the red blood cells of pig and monkey, and only CPV showed agglutination of cat red blood cells. The CPV propagated with variable efficiency in the cultured cells of cat and dog origin, while the FPLV grew only in cat cells. Moreover, the present results demonstrate that CPV was inactivated completely by heating at 80°C for one hour as compared to the inactivation of FPLV at 80°C for 2 hours. Thus the heat stability of these viruses may be an additional criterion for differentiation of FPLV and CPV.

A certain difference in the biological and physical properties between CPV and FPLV have an interesting bearing on the origin of CPV, because it has been suggested that CPV may have originated as a mutant virus from wild-type or live attenuated vaccine strain of FPLV in a veterinary biological product [12, 13]. In this study, therefore, an attempt was made to compare the genomes of CPV and FPLV by restriction enzyme analysis with Bgl II and Hind III of their replicating form DNAs [14]. Our findings indicate a considerable difference at both ends of the DNA between CPV and FPLV, though the 2 fragments of 660 and 900 base pairs in the central part were common to both viral DNAs. In this connection, the restriction site map of CPV was closely related to that of FPLV since about 80% of the 56 maped sites were common for their DNAs [19]. However, a significant correlation between the biological characteristics and the viral genomes of CPV or FPLV is still not known. Such studies are in progress in our laboratories.

After experimental infection with CPV, domestic dogs and cats developed typical symptoms of the disease and high HI titers to
the virus in many cases, and one dog and one cat died at 4 to 5 DPI. In contrary, none of the SPF cats inoculated with CPV showed any clinical signs though they had HI titers of 32 to 512 at the 4th to 11th days. This finding is in accord with the result of other workers [15], but literature dealing with the experimental infection of CPV in domestic and SPF cats is scanty. In our previous study [9], however, the SPF cats inoculated with FPLV showed mild clinical signs such as transitory fever and slight diarrhea throughout the observation period. This is in contrast to the present result obtained with the SPF cats inoculated with CPV. These findings are of great interest in the consideration of the host-virus interaction in CPV infection. The experimental infection of FPLV in domestic dogs is also rare. In the present study, FPLV infection in dogs was performed in 2 cases. As a result, they showed mild clinical signs such as diarrhea, vomiting, or leukopenia, and had high HI serum antibody titers to the virus at 7 to 11 DPI, and FPLV was recovered from their feces on the 6th day. However, further work into aspect of parvovirus infection is needed at various time intervals after the virus inoculation, and now in progress.

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要約

イス・パルボウイルスとネコ汎白血球減少症ウイルスの理化学的ならびに生物学的性状の比較試験：後藤仁・
平野幹泰・内田英二・渡辺清香・品川健一・一条 茂・清水亀平（帯広畜産大学家畜病生物学教室、
家畜内科学教室、畜医公衆衛生学教室）——イス・パルボウイルス（CPV）とネコ汎白血球減少症ウイルス
（FPLV）の理化学的ならびに生物学的性状を比較検討した。両ウイルスの相関な諸性状、形態および大きさでは
差が認められなかったが、熱抵抗性では CPV は 80°C 1 時間、FPLV は 80°C 2 時間の加熱で不活化された。
また、両ウイルスの培養細胞での増殖性と赤血球凝集性に差のあることを確認した。両ウイルスの増殖型ウイルス
DNA の Bgl II と Hind III による制限酵素切断図で、DNA 中央部は両ウイルスに共通であったが、両
端部に違いがみられた。ふつうイスの CPV あるいは FPLV 感染試験とふつうネコの CPV 感染試験では嘔吐、
下痢、白血球減少などの臨床症状がみられたが、SPF ネコの CPV 感染では全く症状が認められなかった。しか
し、ウイルス接種動物では、臨床症状の有無に関係なく、接種 3～11 日目で明らかな血中抗体の上昇が観察さ
れた。