Evaluation of Tri-combinant Vaccine for Feline Herpesvirus, Calicivirus and Panleukopenia Virus Infections in Japanese Native Cats

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Tri-combinant vaccine consisting of attenuated feline herpesvirus (FHV) and feline calicivirus (FCV) and inactivated feline panleukopenia virus (FPLV), were evaluated for safety and efficacy, using Japanese native cats and the viral strains isolated in Japan. Thirty-eight 9- to 12-week-old kittens were inoculated intramuscularly and subcutaneously with the vaccine. Consequently, no adverse reaction was found, and protective efficacy was confirmed by challenge tests with the virulent strains of each virus. Serum-neutralizing antibodies against FCV and FPLV were maintained for at least one year after vaccination, whereas antibody against FHV disappeared in two cases at 24 weeks after vaccination. Application of this vaccine seemed effective for control of feline viral disease in cats for experimental use.

Viral respiratory diseases caused by feline herpesvirus (FHV) and feline calicivirus (FCV), and panleukopenia by feline panleukopenia virus (FPLV) are highly contagious and ubiquitous infections in laboratory cat colonies. They present a major impediment for animal experiments using cats. In order to determine the actual status of FHV and FCV infections in cats for research use and to control them, the authors have conducted a serological and virological survey [19], and showed the efficacy of disinfectants for both these viruses [18].

Vaccines for feline viruses including FHV, FCV and FPLV have been developed and commercialized in several countries [7,14]. In Japan, neither the vaccine for FHV nor FCV has been licenced and commercialized, though one feline vaccine for FPLV is permitted.

The present study was carried out to assure the safety and efficacy of the tri-combinant vaccine for FHV, FCV and FPLV, using Japanese native cats and virus strains isolated in Japan. The application to cats for experimental use was also discussed.

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Materials and methods

Cats: Fifty 9- to 12-week-old Japanese native cats were obtained from households in the vicinity of our university. Each of them was kept in a single cage in the animal rooms at a temperature of 23±1°C with a relative humidity of 55 ±5%. The cats were fed on commercial diets (CS, Oriental Yeast Inc., Tokyo) and canned meat (Dog meal, Hokuetsu Shiryo Inc., Niigata). The cats were sufficiently adapted to laboratory cages by careful rearing for 2 weeks or more before use.

Vaccine and vaccination: The tricombinat vaccine (FVR-CP vaccine, Pitman-Moore Inc., U.S.A.) examined here consisted of attenuated FHV and FCV, and a formalin-inactivated FPLV. The vaccine strains, F-2 of FHV and F-9 of FCV, were attenuated [1] and grown in feline tongue cell line and lyophilized. The formalin-inactivated feline panleukopenia vaccine serving as the diluent for FHV-FCV components was the same vaccine (Feline infectious enteritis vaccine, Pitman-Moore), licenced and commercialized in Japan. These vaccines were imported by Kyoritsu Shoji Inc. (Tokyo) for laboratory use. Each half (0.5ml) of the vaccine was inoculated intramuscularly into the femur and subcutaneously into the neck of each cat. The second vaccination was made at 3 weeks later.

Cell and cell culture: Crandell feline kidney (CRFK) cells were used for the viral propagations and assays. The cells were grown in Eagle’s minimum essential medium (MEM) containing 10% inactivated foetal calf serum (FCS), 100 units of penicillin G potassium and 100 µg of streptomycin sulfate per ml of medium. The maintenance medium was MEM supplemented with 1% of FCS.

Serum-neutralization (SN) test: The antibody assays for FHV, FCV and FPLV were performed by SN test using F-2 strain of FHV, F-9 strain of FCV and TU-1 strain of FPLV. To each 0.3ml of twofold serum dilutions, 0.1ml of each virus suspension (200 TCID₅₀/0.1ml) was added. After incubation at 37°C for 60 min, each 0.1ml of serum-virus mixture was added to two test tubes containing 10⁶ of CRFK cells suspended in 0.5ml of growth medium. The determination of cytopathogenic effect (CPE) associated with FHV or FCV was performed after incubation at 37°C for 7 days. For FPLV antibody assay, hemagglutinating activity (HA) of culture media was examined using 0.5 % swine erythrocyte after incubation for the same period. SN titers were expressed as the reciprocal of the highest dilution inhibiting CPE or HA in one or more of the two tubes tested.

Challenge viruses and challenge method: The viruses used for the challenge test to vaccinated and unvaccinated kittens were C7301 and KS-1 strains of FHV, K-1, 255 and CFI strains of FCV, and TU-1 strain of FPLV. These strains of viruses were virulent, and of them C7301 [12], KS-1 [18], K-1 [8] and TU-1 [11] have been isolated in Japan. At 6 weeks following the initial vaccination, 14 vaccinated kittens and 12 susceptible unvaccinated kittens were challenged with each strain of the viruses grown in CRFK cells. The kittens were anesthetized with ketamine hydrochloride, and given intranasal drops containing approximately 10⁴ TCID₅₀/1ml of FHV or FCV. The same doses of FPLV were suspended in milk and given perorally. These kittens were observed daily for clinical signs.

The challenge tests with each viruses were carried out separately in animal rooms disinfected with formaldehyde before every experiments.

Results

Vaccine safety: Fourteen of kittens were inoculated with the vaccine and clinical signs observed for 6 weeks after initial vaccination, especially in regard to
Table 1. Hematologic examination of vaccinated kittens

<table>
<thead>
<tr>
<th>Items</th>
<th>Pre-vaccination</th>
<th>Post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 weeks</td>
</tr>
<tr>
<td>Red blood cells (x10^6/mm^3)</td>
<td>603±76*</td>
<td>640±101</td>
</tr>
<tr>
<td>White blood cells (x10^6/mm^3)</td>
<td>11.2±2.4</td>
<td>10.8±1.7</td>
</tr>
<tr>
<td>Baso.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eosino.</td>
<td>5.5±2.2</td>
<td>3.6±2.2</td>
</tr>
<tr>
<td>Neutro. (%)</td>
<td>54.7±9.2</td>
<td>59.4±11.5</td>
</tr>
<tr>
<td>Lympho. (%)</td>
<td>34.9±10.5</td>
<td>31.9±11.0</td>
</tr>
<tr>
<td>Mono. (%)</td>
<td>5.0±1.6</td>
<td>5.1±3.5</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>28.3±4.2</td>
<td>27.2±4.4</td>
</tr>
</tbody>
</table>

*M=Mean±SD  n=14

Table 2. Serum-neutralizing antibody titers for FHV, FCV and FPLV in vaccinated kittens

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>SN titers for FHV</th>
<th>SN titers for FCV</th>
<th>SN titers for FPLV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-vacci.</td>
<td>Post-vacci. (weeks)</td>
<td>Pre-vacci.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
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<tr>
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<td>2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>4</td>
<td>&lt;2</td>
<td>8</td>
<td>&lt;2</td>
</tr>
<tr>
<td>5</td>
<td>&lt;2</td>
<td>8</td>
<td>&lt;2</td>
</tr>
<tr>
<td>6</td>
<td>&lt;2</td>
<td>2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>7</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
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<td>8</td>
<td>&lt;2</td>
<td>4</td>
<td>&lt;2</td>
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<tr>
<td>9</td>
<td>&lt;2</td>
<td>8</td>
<td>&lt;2</td>
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<tr>
<td>10</td>
<td>8</td>
<td>4</td>
<td>&lt;2</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>4</td>
<td>&lt;2</td>
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<td>12</td>
<td>8</td>
<td>16</td>
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<tr>
<td>14</td>
<td>4</td>
<td>2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

SN titers were expressed as reciprocal of the highest dilution inhibited CPE (FHV, FCV) or HA (FPLV) in one or more tubes of two tested.

general conditions (appetite, rectal temperature, body weight), local reactions of inoculating sites (pain, swelling, induration) and general reactions (conjunctivitis, rhinitis, respiratory sign, oral ulceration, dehydration, vomiting, diarrhea, shock). Adverse effects were not observed in any of the 14 vaccinated kittens. Blood speci-mens were obtained from the vaccinated kittens at the time of pre-vaccination and at 3 and 6 weeks after initial vaccination, and submitted to hematologic examinations. The result is shown in Table 1. Total counts of erythrocyte and leukocyte, leukogram and packed cell volume (PCV) remained well within normal limits.
Table 3. Viral challenge for vaccinated and unvaccinated kittens

<table>
<thead>
<tr>
<th>Group</th>
<th>Challenge viruses</th>
<th>Strains</th>
<th>SN titers at challenge</th>
<th>Clinical signs</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>FHV</td>
<td>C7301</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C7301</td>
<td>4</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>C7301</td>
<td>&lt;2</td>
<td>+</td>
<td>Cured</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KS-1</td>
<td>8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>FCV</td>
<td>K-1</td>
<td>128</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K-1</td>
<td>128</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K-1</td>
<td>32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CFI</td>
<td>128</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>255</td>
<td>32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>FPLV</td>
<td>TU-1</td>
<td>640</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TU-1</td>
<td>160</td>
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<td></td>
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<td>80</td>
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<td></td>
<td>TU-1</td>
<td>&lt;10</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>FHV</td>
<td>C7301</td>
<td>&lt;2</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C7301</td>
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<td></td>
<td></td>
<td>C7301</td>
<td>&lt;2</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KS-1</td>
<td>&lt;2</td>
<td>+</td>
<td>Sacrificed</td>
</tr>
<tr>
<td></td>
<td>FCV</td>
<td>K-1</td>
<td>&lt;2</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K-1</td>
<td>&lt;2</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>K-1</td>
<td>&lt;2</td>
<td>+</td>
<td>Cured</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CFI</td>
<td>&lt;2</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td></td>
<td>255</td>
<td>&lt;2</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td>FPLV</td>
<td>TU-1</td>
<td>&lt;10</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TU-1</td>
<td>&lt;10</td>
<td>+</td>
<td>Dead</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TU-1</td>
<td>&lt;10</td>
<td>+</td>
<td>Dead</td>
</tr>
</tbody>
</table>

10^4 TCID_{50} of each viruses was given intranasally (FHV, FCV) and perorally (FPLV).

Antibody responses: The antibody responses following vaccination are presented in Table 2. At 6 weeks after initial vaccination, SN antibodies ranging from 1:4 to 1:32 (average 1:9) against FHV, 1:16 to 1:512 (average 1:95) against FCV, and 1:80 to 1:2,560 (average 1:470) against FPLV, were developed in all of the vaccinated kittens with the exception of the kittens with an antibody appeared to be maternally derived. Sero-positive kittens at the time of vaccination have not developed typical antibody response, but showed the declivity of antibody titers. SN antibody against FHV was developed in lower titers than that against other components.

Protective responses: To compare the protective responses between these vaccinated and unvaccinated kittens, the challenge with the viruses was performed at 6 weeks after initial vaccination. The results are shown in Table 3. Four of 5 vaccinated kittens remained well after FHV challenge exposure, whereas all 4 unvaccinated kittens developed definite
signs of FHV disease, sneezing, nasal and ocular discharge, salivation and fever. They were dead or sacrificed at the moribund stage at 9 to 14 days after the challenge. One of the vaccinated kittens had no detectable antibody at the time of challenge, under the influence of maternal immunity. This one showed slight clinical signs from 5 to 13 days after the challenge, but was cured. All 5 vaccinated kittens challenged with 3 strains of FCV showed no clinical sign. On the contrary, the controls challenged with the same viruses developed typical respiratory signs, and died at 12 to 18 days after the challenge except one. After the challenge exposure of FPLV, 3 of 4 vaccinated kittens remained healthy, but one of them and 3 controls died at 5 to 13 days, developing fever, diarrhea and vomiting. The one which died in spite of the vaccination had no detectable antibody against FPLV, when exposed to the challenge.

**Duration of immunity**: A total of 24 kittens were vaccinated by the same method described above, and confined in the animal room for a 1-year period. They were observed daily for clinical signs and weighed weekly. Moreover, the sera from 12 of them were offered repeatedly for assays of SN antibodies, in order to observe the duration of immunity. The persistence of antibody titers following vaccination is illustrated in Fig. 1. FHV antibody titers already declined at 12 weeks following the peak of $2^{14}$ at 6 weeks after vaccination. Thereafter FHV antibody became undetectable in two cases at 24 weeks after vaccination. Both respective antibody titers against FCV and FPLV were maintained at approximately $2^{1.8}$ to $2^{2.2}$ and $2^{3.7} \times 10$ to $2^{4.9} \times 10$ for 1 year after vaccination.

All 24 kittens grew up well, showing no clinical sign of infection with FHV, FCV or FPLV for 1 year of observation period. Body weights of these vaccinated cats reached to approximately 3.2 to 3.7kg (male) and 2.4 to 3.1kg (female) at the end of observation.

**Discussion**

The F-2 strain of FHV and the F-9 strain of FCV were selected and attenuated by Bittle and York [1] as the viral strains of choice for vaccine development. It has been indicated that these strains did not cause significant disease and retained their antigenicity when administered intramuscularly [2,3,4,17]. Concerning the antigenic difference of FCV, the F-9 strain was found to be serologically related to all other strains tested [10], and induced antibodies against many strains including highly virulent 255 strain [5,9]. The formalin-inactivated feline panleukopenia vaccine serving as the diluent for lyophilized FHV and FCV was the same one already licenced and commercialized here in Japan.

The results of our observation of clinical signs and hematologic examination indicated that the vaccine was safe and
free of any adverse reactions when administered intramuscularly and subcutaneously to healthy cats. Exceptions for the fact that some had maternal immunity when they were vaccinated, all the vaccinated kittens developed typical antibody response against each virus. They showed no clinical signs of infections with FHV, FCV or FPLV when challenge exposed. Thus, the present study ensured the safety and efficacy of the vaccine for Japanese native cats and the viral strains isolated in Japan.

The maternal antibodies against FHV and FCV have been shown to be retained for 9 to 10 weeks after birth [18]. When the queens had high antibody titers against FPLV, the kittens kept their maternal antibody as long as 12 to 16 weeks [16]. The maternal antibodies seemed to interfere with the efficacy of the vaccine in some cases presented here, even though the kittens were vaccinated initially at 9 to 12 weeks of age and revaccinated at 3 weeks interval. The absence of maternal immunity must be confirmed to assure good antibody production in kittens.

Bittle and Rubic have reported that vaccinated cats had low titers against FHV at 1 year after vaccination were protected when challenge exposed [4]. The results of this study showed that SN antibody titer against FHV was lower and declined earlier than that against other viruses, but could protect against FHV challenge. The protective immunity against FHV may be concerned not only with humoral antibody but also with local and cell-mediated response. Thus, vaccinated cats appeared to have sufficient protective immunities at least for 6 months against FHV challenge and 1 year against FCV and FPLV.

As for the viral shedding from vaccinated cats, it has been reported that vaccinal viruses were not shed to susceptible contact controls [17]. So far as the vaccine is injected intramuscularly or subcutaneously, it will be not need to fear on the shedding of vaccinal virus. Orr et al. [13,15] have described about the interaction of the attenuated live vaccine such as the vaccine presented here with FHV carriers. The problem, however, whether or not should vaccinate for carriers seemed to be not concluded. As it is a considerable problem, the persistence and excretion of virus after vaccination on FHV carriers must be determine in future studies.

On the other hand, the authors have showed that the mature cats for experimental use obtained from dealers and a pound have frequently included carriers of FHV and FCV in previous report [19]. Vaccination may not show uniform effects on these cats, because they have various infective agents and are in various stages of infection including carrier state. Therefore, these mature cats must be isolated individually with equipment such as the isolate-rack which can regulate the air flow to be negative [6], and used for short-term experiments. Tri-combiant vaccine examined here is considered to be very useful especially for kittens from households, as there is little possibility that they include the carrier cats. The kittens vaccinated and examined for the duration of immunity in this study were employed for long-term neurophysiological experiments.

There has been a general tendency to avoid vaccination of laboratory animals, because of the influence on experiments, especially in the fields of immunology and microbiology. Cats, however, are employed mainly for experiments in the fields of neurophysiology, pharmacology and ethology. Therefore, vaccination may well have little or no influence on these experiments. Vaccination should be carefully undertaken to prevent viral diseases in cats for experimental use.

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References

ネコヘルペスウイルス、カリシウイルス、および汎白血球減少症ウイルス感染に対する３種混合ワクチンの評価

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弱毒化したネコヘルペスウイルス（FHV）、ネコカリシウイルス（FCV）および不活化したネコ汎白血球減少症ウイルス（FPLV）を含む３種混合ワクチンの安全性およびその効力をについて、日本在来のネコとわが国で分離されたウイルス株を用いて検討した。9-12週齢の健康な幼齢ネコ38頭の筋肉内および皮下に、本ワクチンを接種し、3週後に再接種を行なった。その結果、副作用もなく安全で、それぞれのウイルスによる攻撃に対して、充分な防御効果をもつことが確認された。また、ワクチン接種ネコの FCV および FPLV に対する中和抗体は、1年以上持続したが、FHV に対する中和抗体は、一部のネコで接種後24週で消失した。実験用ネコのウイルス性疾患を防ぐために、本ワクチンの応用是有効な手段と思われる。