Neutralizing Antibodies against Feline Parvoviruses in Nondomestic Felids Inoculated with Commercial Inactivated Polyvalent Vaccines

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(Received 28 December 2005/Accepted 6 July 2006)

ABSTRACT. The virus neutralization (VN) antibody titers of serum samples from 18 individuals representing 8 carnivore species vaccinated with commercial polyvalent vaccines optimized for domestic cats containing inactivated feline panleukopenia virus (FPLV) were evaluated against canine parvovirus type 2 (CPV2). In addition, the titers among 5 individuals from 4 carnivore were evaluated against antigenic variants of feline parvoviruses; FPLV, CPV2, CPV2a, CPV2b, CPV2c, mink enteritis virus type 1 (MEV1) and MEV2. The polyvalent vaccines induced cross-reactive VN titers against antigenic variants of feline parvoviruses in nondomestic felids. However, we observed very low cross-reactive VN antibody in lions and Siberian tigers, therefore we should pay attention to CPV infections in these animals even if they were vaccinated with inactivated FPLV vaccines.

KEY WORDS: felids, parvovirus, vaccine.

Feline panleukopenia virus (FPLV), mink enteritis virus (MEV) and canine parvovirus (CPV) are classified as the feline parvovirus subgroup of the genus Parvovirus family Paroviridae. FPLV was first recognized in domestic cats (Felis catus) in 1928 [33]. In 1978, CPV type 2 (CPV2) emerged in domestic dogs and spread. Genetic drift soon changed its antigenicity [24]. CPV2a appeared in 1980 or 1981, and CPV2b in 1984, both of whose host ranges now included cats [20]. CPV2c was isolated from leopard cats (Prionailurus bengalensis) in Southeast Asia in 1997 [14] and has pathological potential in domestic cats [22]. Although there are many hypotheses regarding this expansion of host ranges, the involvement of wild animals as mediators is the most widely accepted explanation [32]. Prevention of parvovirus infections in zoos is important not only for protecting precious and endangered species from serious diseases, but also for prohibiting host range expansion.

The immunization of nondomestic feline animals against feline parvoviruses has been recommended [7]. As vaccines for nondomestic felids are not available commercially, animals have been vaccinated with inactivated FPLV vaccines for domestic cats as substitutes. The safety and efficacy of an inactivated polyvalent vaccine containing FPLV for use in domestic cats commercialized in the U.S. was tested by measuring virus neutralization (VN) titers among nondomestic felids vaccinated with one dose (1 ml) and one injection [2]. However there are no reports about cross immunity against new antigenic types. The causative antigenic types reported in nondomestic felids so far are FPLV, CPV2a and CPV2b, and the dominant parvovirus antigenic type was reported to be CPV’s not FPLV [30]. Indeed, deaths from CPV2b infection were reported in April 2005, among four cheetahs vaccinated with inactivated FPLV vaccines [30]. In this study we tried to clarify the efficacy of vaccinations against antigenic types of parvovirus, and observed cross-reactive VN activities against several antigenic types of parvovirus among vaccinated captive nondomestic felids kept in a zoo.

Serum samples from nondomestic felids were obtained from captive animals kept in Asahikawa Municipal Asahiyama Zoological Park and Wildlife Conservation Center (lat. 43° 46’ N. and long. 142° 29’ E., 170 meters above sea level). All felids used in this study were vaccinated with polyvalent vaccines containing FPLV, feline calicivirus (FCV) and feline herpesvirus (FHV) at least once. The stored serum samples were taken from 1999 to 2004 from 18 individuals representing 8 species; 2 Siberian tigers (Panthera tigris altaica), 3 Amur leopards (Panthera pardus orientalis), a black leopard (Panthera pardus), 2 snow leopards (Panthera uncia), 4 lions (Panthera leo), 4 clouded leopards (Neofelis nebulosa), an ocelot (leopardus pardalis) and a Siberian lynx (Lynx lynx). From April to June 2005, blood samples (5 ml) were newly obtained from 5 individuals covering 4 species; a lion, a clouded leopard, an Amur leopard, and 2 Siberian tigers.

feline kidney (CRFK) cells (a feline kidney cell line) [5], Madin-Darby canine kidney (MDCK) cells (a canine kidney cell line) [9] or 3201 cells (a feline lymphosarcoma cell line) [29]. The viruses were inoculated onto appropriate cells, and the cytopathic effect (CPE) was observed. After three cycles of freezing and thawing, culture supernatants were passed through 0.45 µm filters (Millipore, Bedford, MA, U.S.). The viruses were titrated on FL74 cells (a feline T-lymphoblastoid cell line) [31] according to the method established by Ikeda et al. [13] with slight modification; instead of using 1 × 10^4 cells/ml of FL74, 1 × 10^5 cells/ml was used as indicator cells. The 50% tissue culture infectious dose (TCID_{50}) was determined using the Kärber method [16]. VN tests were performed according to the method described by Ikeda et al. [13] with slight modification. Briefly, 2-fold serially diluted serum samples (50 µl) were mixed with solutions (50 µl) of the virus (100 TCID_{50}), and incubated at 37°C for 1 hr. Then FL74 cells (100 µl, 2 × 10^5) were added.

The VN titers of stored sera and newly obtained sera are shown in Tables 1 and 2, respectively. The stored sera were examined in terms of their VN titers against CPV2 strain Cp49. Relatively low titers were observed in 3 (1: 12, 1: 14, 1: 34) of 4 lions, and one (1: 67) of 2 snow leopards (Table 1). Other animals showed titers ranging from 1: 135 to more than 1: 7,241, even though it had been more than 10 months since the final vaccination in most animals. There was no relationship between the VN titer and the period post-vaccination. The VN titers of newly obtained sera were relatively high (>1: 113) against all antigenic types tested except in one Siberian tiger and one lion who showed relatively low titers (1: 57) against two CPV2c strains V139 and V140, respectively (Table 2). One clouded leopard and one Amur leopard showed titers of over 1:7,241 against all antigenic types tested. In the Siberian tigers and one lion, the VN titers against CPV2c were significantly lower than those for the other antigenic types (P<0.05, Kruskal-Wallis test).

Although zoos in Japan are usually under very strict quarantine regulations, the viruses are ubiquitous and the risk of exposure is relatively high. Feline parvoviruses are contagious and very resistant in environments that can not be inactivated by most disinfectants: in fact, chloride is the only disinfectant that inactivates parvoviruses [27]. In addition, the majority of felids are suggested to be susceptible to FPLV [4]. Once an outbreak of parvovirus infections has occurred, elimination of the pathogens from the zoo is difficult. Preventing an outbreak is preferable, and vaccination using commercial polyvalent vaccines for domestic cats is thought to be one of the most effective ways to protect nondomestic felids from parvovirus infections. To determine the efficacy of vaccinations against feline parvoviruses in nondomestic felids, experiments should ideally include the challenge of vaccinated nondomestic felids with pathogenic feline parvoviruses, however, the preciousness of the animals and the risks involved make this impossible. In this study, the VN titer was used as an indicator of protection.

### Table 1. Profiles of stored serum samples and VN titers

<table>
<thead>
<tr>
<th>Animals</th>
<th>ID No.</th>
<th>VN titers</th>
<th>Dates of extraction</th>
<th>Vaccination records</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siberian tiger</td>
<td>1α)</td>
<td>453</td>
<td>2004-Dec.24</td>
<td>1 68 months</td>
</tr>
<tr>
<td></td>
<td>1α)</td>
<td>381</td>
<td>2002-Nov.13</td>
<td>1 42 months</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6,089</td>
<td>2004-Mar.23</td>
<td>2 16 months</td>
</tr>
<tr>
<td>Amur leopard</td>
<td>1</td>
<td>&gt;7,241</td>
<td>2002-Oct.19</td>
<td>1 41 months</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>538</td>
<td>2004-Dec.13</td>
<td>2 24–35 monthsα)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;7,241</td>
<td>2004-Dec.14</td>
<td>1 44 months</td>
</tr>
<tr>
<td>Black leopard</td>
<td>1</td>
<td>135</td>
<td>2004-Dec.15</td>
<td>1 44 months</td>
</tr>
<tr>
<td>Snow leopard</td>
<td>1</td>
<td>160</td>
<td>2004-Dec.16</td>
<td>1 67 months</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>67</td>
<td>2004-Oct.20</td>
<td>1 65 months</td>
</tr>
<tr>
<td>Lion</td>
<td>1</td>
<td>1,810</td>
<td>2004-Dec.14</td>
<td>1 11–23 monthsβ)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>34</td>
<td>2004-Dec.13</td>
<td>2 33 months</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>2004-Jun.26</td>
<td>2 25 months</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>14</td>
<td>2002-Mar.17</td>
<td>1 7 months</td>
</tr>
<tr>
<td>Clouded leopard</td>
<td>1</td>
<td>&gt;7,241</td>
<td>2001-Apr.21</td>
<td>Unknownβ)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;7,241</td>
<td>2001-Apr.24</td>
<td>Unknownβ)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;7,241</td>
<td>2004-Dec.17</td>
<td>Unknownβ)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>&gt;7,241</td>
<td>2001-Aug.29</td>
<td>1 4 months</td>
</tr>
<tr>
<td>Ocelot</td>
<td></td>
<td>&lt;7,241</td>
<td>2001-Apr.20</td>
<td>1 22 months</td>
</tr>
<tr>
<td>Siberian lynx</td>
<td>1,280</td>
<td>1999-May 19</td>
<td>1 42 months</td>
<td></td>
</tr>
</tbody>
</table>

α) Siberian tiger 1 was tested twice as the extraction dates were different.
β) Three clouded leopards were vaccinated but the dates of vaccination are unknown.
γ) Details are unknown.
Protection against antigenic types of feline parvovirus by immunizing with different antigenic types was reported in domestic cats [3, 8], domestic dogs [11, 25, 26] and minks [10, 23, 34]. In the present study, we observed cross-reactive VN antibodies in nondomestic felids vaccinated with the inactivated FPLV vaccine. Reportedly, a VN titer of 1:8 could prevent FPLV infection [6, 17]. However, VN titers could be varied depending on the method of the VN tests. We can not exclude the possibility that our VN assay system using FL74 cells is far more sensitive to VN antibodies than their assay systems. Therefore, we can not define clearly the VN antibody titer which protects animals from parvovirus infection. We found that some lions had very low VN antibody titers (1:12 to 1:34) against CPV2 strain CP49. In addition, relatively low VN titers against CPV2c were observed in freshly isolated sera from Siberian tigers and a lion. This result is consistent with our previous paper [21], where the cross-reactive VN activity against CPV2c was lower than that against other antigenic types. Surprisingly, titers of more than 1:7,241 were observed even in felids vaccinated 44 months ago (Table 1). Although no animal in the zoo has ever shown any clinical signs of a feline parvovirus infection, it is possible that nondomestic felids were naturally boosted with feline parvoviruses in the field. Together with the observation of low cross-reactive VN antibody in lions and Siberian tigers, we should pay attention to CPV infections in nondomestic feline animals even if they were vaccinated with inactivated FPLV vaccines.

ACKNOWLEDGEMENTS. We thank Dr. Y. Tohya (The University of Tokyo, Bunkyo-ku, Tokyo, Japan) for providing the feline paroviruses used in this study. We are grateful to Dr. M. Mochizuki (Kyoritsu Seiyaku Co., Chiyoda-ku, Tokyo, Japan) for valuable advice. This study was partly supported by a contribution from Kyoritsu Seiyaku Corporation.

REFERENCES