Effect of vaccination on parvovirus antigen testing in kittens

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Objective—To determine the frequency and duration of feline panleukopenia virus (FPV) vaccine-induced interference with fecal parvovirus diagnostic testing in cats.

Design—Prospective controlled study.

Animals—Sixty-four 8- to 10-week-old specific-pathogen–free kittens.

Procedures—Kittens were inoculated once with 1 of 8 commercial multivalent vaccines containing modified-live virus (MLV) or inactivated FPV by the SC or intranasal routes. Feces were tested for parvovirus antigen immediately prior to vaccination, then daily for 14 days with 3 tests designed for detection of canine parvovirus. Serum anti-FPV antibody titers were determined by use of hemagglutination inhibition prior to vaccination and 14 days later.

Results—All fecal parvovirus test results were negative prior to vaccination. After vaccination, 1 kitten had positive test results with test 1, 4 kittens had positive results with test 2, and 13 kittens had positive results with test 3. Only 1 kitten had positive results with all 3 tests, and only 2 of those tests were subjectively considered to have strongly positive results. At 14 days after vaccination, 31% of kittens receiving inactivated vaccines had protective FPV titers, whereas 85% of kittens receiving MLV vaccines had protective titers.

Conclusions and Clinical Relevance—Animal shelter veterinarians should select fecal tests for parvovirus detection that have high sensitivity for FPV and low frequency of vaccine-related test interference. Positive parvovirus test results should be interpreted in light of clinical signs, vaccination history, and results of confirmatory testing. Despite the possibility of test interference, the benefit provided by universal MLV FPV vaccination of cats in high-risk environments such as shelters outweighs the impact on diagnostic test accuracy. (J Am Vet Med Assoc 2007;230:359–363)

Although widespread vaccination of pet cats has made FPV infection an uncommon diagnosis at present, animal shelters continue to report large-scale outbreaks of FPV.1-10 Feline panleukopenia virus is a highly contagious parvovirus of cats that is fatal in 30% to 90% of untreated cats and is the most frequent cause of death in cats in animal shelters.11-13 The intensive housing of cats in shelters and durability of the virus contribute to rapid spread of FPV within susceptible cat populations. Outbreaks commonly occur in the summer and fall following a large influx of kittens born in the spring that are admitted to shelters at an age when maternal immunity is waning.10 Kittens are understood to be the most susceptible to FPV infection, but shelter outbreaks may also involve adult cats. Because the incubation period after exposure is 2 to 14 days, apparently healthy cats may leave the shelter for adoption or fostering, only to succumb to infection in their new homes. Institutional responses to outbreaks frequently include temporary closure to new cat admissions or depopulation of entire cat populations, even those without evidence of infection.1-10

The primary route of FPV transmission is fecal-oral contamination. After infection, FPV primarily infects rapidly dividing cells of the bone marrow, lymphoid system, and intestinal mucosa, resulting in severe panleukopenia, vomiting, diarrhea, hypovolemic shock, sepsis, and death. Less commonly, a peracute syndrome occurs in which cats become moribund and die within hours, without manifesting typical gastrointestinal signs. Subclinical disease in partially resistant cats may be accompanied by modest leukopenia in the absence of obvious clinical signs. Kittens infected in utero near term or in the early postnatal period may have viral replication in the granular layer of the cerebellum, causing permanent cerebellar dysfunction and ataxia. Early in utero infection often results in fetal death. High con-

**Abbreviations**

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>FPV</td>
<td>Feline panleukopenia virus</td>
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<tr>
<td>MLV</td>
<td>Modified-live virus</td>
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<td>SPF</td>
<td>Specific-pathogen–free</td>
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centrations of FPV may be shed in feces prior to the onset of clinical signs and for several weeks after recovery, although shedding is limited to a 5- to 7-day period in most instances. Feline panleukopenia virus is extremely resistant to inactivation, with most disinfectants other than sodium hypochlorite, potassium peroxymonosulfate, formaldehyde, and glutaraldehyde having little effect on the virus. Infectious viral particles can survive in the environment for longer than 1 year.19-17 Point-of-care fecal antigen test kits are available to confirm parvovirus infection in dogs within a few minutes, allowing rapid identification and isolation of dogs that are shedding parvovirus in feces.18 These canine parvovirus tests also detect FPV antigen in cat feces, although this is not a licensed use and the sensitivity and specificity of these tests in cats have not been reported.19-22 Other laboratory-based diagnostic tests, including virus isolation, PCR assays, and seroconversion, may be more accurate for parvovirus detection, but rapid-assay point-of-care tests are more practical and cost-effective for quick screening of animals in the shelter setting. Thus, although canine parvovirus tests are not validated for use in cats, they are commonly used in this species by shelter veterinarians.

Highly effective inactivated and MLV vaccines are available for immunization against FPV.23-25 Modified-live virus vaccines offer rapid-onset protection, which is essential for preventing FPV transmission in shelters. In seronegative cats, primary immunization against FPV with MLV vaccines results in detectable serum antibodies in 5 to 7 days, but confers protection against infection even earlier. In 1 study,26 susceptible kittens could be safely housed in a contaminated environment immediately after vaccination, and full protection against highly virulent challenge was reached by 72 hours after vaccination. In contrast, use of inactivated vaccines results in a slower immune response, often requiring several weeks before protective serum antibody titers are reached.24 Immunization is less effective in kittens with passively acquired maternal antibodies against FPV. Vaccine interference by those antibodies has been detected up to 19 weeks of age.19,27,28

The high number of susceptible cats housed in shelters, durability and high transmission rates of FPV, and high fatality rates among infected cats mandate the use of vigorous preventive measures to protect the health of cats in shelters. Preventive health guidelines for shelters now urge immunization with MLV FPV vaccines immediately upon cats’ admission to the shelter.23-25 Vaccination of all cats, regardless of their potential for eventual adoption, is recommended to improve the resistance of the entire shelter population and to reduce environmental contamination by FPV. Vaccination of all cats and dogs immediately upon admission to a shelter reduces both the number and severity of feline and canine parvovirus outbreaks in shelters.13,22 However, in dogs, it is believed that parvovirus vaccination sometimes results in transient fecal shedding of vaccine antigens that cannot be distinguished from natural infection with diagnostic test kits.18 If this is also true for cats, recently vaccinated cats might have false-positive test results with fecal parvovirus antigen tests, thus complicating the ability of shelter personnel to test and segregate infected populations. Although this would be true for cats at all types of facilities, it is especially problematic at shelters where large numbers of cats are likely to be both recently vaccinated and manifesting clinical signs such as vomiting and diarrhea, which may or may not be a result of FPV infection.

The purpose of the present study was to determine the frequency and duration of vaccine-induced interference with fecal parvovirus antigen tests in cats by use of 8 commercially available vaccines and 3 canine parvovirus tests.

Materials and Methods

Cats—Eleven SPF queens and their 64 kittens were enrolled in the study. Seven of the queens had previously been vaccinated against FPV and were seropositive for antibodies against FPV. Four of the queens were vacci- nated and were seronegative for anti-FPV antibodies. Because passively acquired anti-FPV antibodies inhibit replication of FPV and subsequent fecal antigen shedding, an effort was made to minimize transfer of maternal FPV antibodies to kittens. To optimize the chance of detecting FPV vaccine-derived antigens in feces, kittens of vaccinated queens were colostrum deprived so that they would have negligible serum titers of anti-FPV antibodies at the time of vaccination. Kittens born to seronegative queens were allowed to nurse normally. Kittens were randomly allocated into 8 groups of 8 kittens each when they were 8 to 10 weeks old such that littersmates were distributed among the groups and each group contained 4 males and 4 females. Kittens were housed individually, and staff wore barrier gowns, booties, and gloves beginning the day before vaccination (day –1) to prevent cross-contamination with vaccine viruses. All kittens remained healthy during the study and were seronegative for FeLV antigen and FIV antibodies when tested by means of an ELISA. The research protocol was approved by the University of Florida Institutional Animal Care and Use Committee and was conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Kittens were adopted into private homes at the conclusion of the study.

Vaccination—Eight multivalent vaccines for FPV, feline herpesvirus-1, and feline calicivirus were selected for testing. The vaccines included 5 parenterally administered MLV vaccines, 1 parenterally administered inactivated vaccine, 1 parenterally administered vaccine in which the FPV was inactivated and the herpesvirus-1 and calicivirus were MLVs, and 1 mucosally administered MLV vaccine.34 Vaccines were administered SC in the left hind limb or intranasally as recommended by the manufacturers.

FPV antibody titers—Blood was collected via jugular venipuncture for determination of anti-FPV antibody titers immediately prior to vaccine administration (day 0) and again 14 days later (day 14). Blood was placed in serum separator tubes, allowed to clot for a minimum of 30 minutes, and centrifuged for 10 minutes. Serum was stored at –20°C pending analysis. End point anti-FPV antibody titers were determined via hemagglutination inhibition by use of 2-fold serum di-
lutions starting at 1:10. A titer of 1:40 was considered to be protective against infection.29

Parvovirus antigen testing—Fecal samples were collected once daily from the litter box of each kitten for 15 days beginning immediately prior to administration of the vaccines. If there were no feces in the litter box, samples were collected by use of rectal swabs. Immediately after collection, each fecal sample was tested for parvovirus antigen with each of 3 canine parvovirus point-of-care test kits according to manufacturers’ instructions. Each test kit was also used to test feces from a cat with confirmed FPV infection. Finally, the ability of the test kits to detect the FPV antigen contained in the vaccines was determined by use of swabs soaked in each of the vaccines.

Statistical analysis—The proportions of positive parvovirus antigen test results for each of the 3 test kits, for each individual vaccine, for vaccines grouped by inactivated versus MLV FPV, and for vaccines grouped by parenteral versus mucosal administration were calculated and compared by use of the Fisher exact test. Mean FPV antibody titers for each vaccine group, vaccines grouped by inactivated versus MLV, and vaccines grouped by parenteral versus mucosal administration were calculated and compared by use of the Kruskal-Wallis analysis of variance on ranks test. The magnitudes of FPV antibody titers prior to and 14 days after vaccination were correlated with the occurrence of positive test results and compared with the Spearman rank order test. Values of $P < 0.05$ were considered significant.

Results

All 3 fecal parvovirus tests yielded strong positive results when feces from a confirmed FPV-infected cat were tested. Results when vaccines were used as the test samples varied. Test 1 yielded positive results with vaccines 1, 2, 3, 6, 7, and 8. Test 2 yielded positive results with vaccines 1, 2, 6, 7, and 8. Test 3 yielded no positive reactions when vaccines were used as test samples.

Fecal parvovirus antigen testing—All kittens had negative results of fecal parvovirus antigen tests prior to vaccination. When all test results were considered, 13 kittens (20%) had at least 1 positive test result (Table 1). Only 1 kitten had positive test results with all 3 tests, and 3 kittens had positive results with 2 of 3 tests. Individual kittens had positive test results on 1 to 8 testing days with occasional negative test days in between the positives. Test 1 was positive in 1 kitten in 4 consecutive test days (from day 7 through day 10); this kitten had received vaccine 5. Test 2 yielded a positive result in 4 kittens for a total of 8 kitten test days (from day 7 through day 14); positive results occurred in kittens that received vaccines 1, 2, and 5 (2 kittens). Test 3 was positive in all 13 kittens for a total of 24 kitten test days (from day 1 through day 14); positive results occurred with each vaccine except vaccine 8. One kitten that received vaccine 1 and was evaluated with test 3 had 8 days of positive test results interspersed with 4 days of negative test results, from day 3 to day 14. The proportion of positive test results was significantly greater for test 3 for both number of kittens ($P < 0.02$) and number of days with positive test results ($P < 0.004$), compared with test 1 or test 2. Test 1 and test 2 were not significantly different ($P = 0.1$). Of the 36 individual positive test results, only 2 (from the same kitten) were subjectively considered to be strongly positive. This occurred with test 3 in a kitten that received vaccine 1 (an inactivated vaccine). There were no significant differences in the proportion of kittens with positive test results between vaccine groups.

FPV antibody titers—None of the kittens had protective serum antibody titers against FPV prior to vaccination. Eleven kittens (17%) had a low titer of 1:10; these kittens were evenly distributed among the vaccine groups. The remaining kittens were seronegative at the time of vaccination. At 14 days after vaccination, 31% (5/16) of kittens receiving inactivated vaccines had protective titers, whereas 85% (41/48) of kittens receiving MLV vaccines had protective titers ($P < 0.001$; Table 1). In all 7 of the kittens that failed to develop protective titers after receiving MLV vaccines, test results for serum antibodies were negative both prior to and after vaccination. Mean titers 14 days after vaccination were significantly ($P < 0.001$) lower in the kittens that received inactivated vaccines (mean ± SD titer, 28 ± 24 Rodricks) compared with the kittens that received MLV vaccines (mean ± SD titer, 100 ± 38 Rodricks).

Table 1—Serum antibody responses and results of fecal parvovirus antigen testing in 64 kittens that received 1 of 8 FPV vaccines. Feces were tested with 3 fecal canine parvovirus antigen test kits immediately prior to vaccination and daily for 14 days after vaccination. Serum anti-FPV antibody titers were measured immediately prior to vaccination and 14 days later and are expressed as mean ± SD. Titters ± 1:40 were considered protective.

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Vaccine type</th>
<th>Administration route</th>
<th>FPV antibody pre</th>
<th>FPV antibody post*</th>
<th>Percentage protective pre</th>
<th>Percentage protective post*</th>
<th>No. kittens (No. kitten test days) with positive results for FPV on fecal tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>IA</td>
<td>SC</td>
<td>4 ± 5</td>
<td>38 ± 28</td>
<td>0</td>
<td>38</td>
<td>1 (1) (1) (10)</td>
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<tr>
<td>2*</td>
<td>IA</td>
<td>SC</td>
<td>1 ± 4</td>
<td>19 ± 15</td>
<td>0</td>
<td>25</td>
<td>0 (1) (2) (1)</td>
</tr>
<tr>
<td>3*</td>
<td>MLV</td>
<td>SC</td>
<td>1 ± 4</td>
<td>1,766 ± 2,130</td>
<td>75</td>
<td>0</td>
<td>0 (0) (2) (3)</td>
</tr>
<tr>
<td>4*</td>
<td>MLV</td>
<td>SC</td>
<td>1 ± 4</td>
<td>1,766 ± 1,481</td>
<td>100</td>
<td>0</td>
<td>0 (0) (1) (1)</td>
</tr>
<tr>
<td>5*</td>
<td>MLV</td>
<td>SC</td>
<td>4 ± 5</td>
<td>1,996 ± 1,739</td>
<td>0</td>
<td>88</td>
<td>1 (4) (5) (5)</td>
</tr>
<tr>
<td>6*</td>
<td>MLV</td>
<td>SC</td>
<td>1 ± 4</td>
<td>580 ± 483</td>
<td>88</td>
<td>0</td>
<td>0 (0) (1) (1)</td>
</tr>
<tr>
<td>7*</td>
<td>MLV</td>
<td>SC</td>
<td>1 ± 4</td>
<td>1,068 ± 807</td>
<td>88</td>
<td>0</td>
<td>1 (4) (5) (5)</td>
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<tr>
<td>8*</td>
<td>MLV</td>
<td>IN</td>
<td>0 ± 0</td>
<td>1,046 ± 1,023</td>
<td>75</td>
<td>0</td>
<td>0 (0) (0) (0)</td>
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*Serum anti-FPV titers and proportion of kittens with protective titers were significantly ($P < 0.001$) higher 14 days after vaccination in kittens that received MLV vaccines, compared with those in kittens that received IA vaccines. †Proportions of positive test results were significantly ($P < 0.02$) greater for test 3 than for tests 1 or 2.

IA = Inactivated. IN = Intranasal. Pre = Before vaccination. Post = 14 days after vaccination.
than those in kittens that received MLV vaccines (1,265 ± 1,385). The proportion of kittens protected by the mucosally administered MLV vaccine (75% [6/8]) was not significantly different than the proportion of kittens protected by the 5 parenterally administered MLV vaccines (88% [35/40]; P = 0.4). Additionally, mean titers in kittens that received the mucosally administered vaccine (1,040 ± 1,023) were not significantly different than those in kittens that received the parenterally administered MLV vaccines (1,310 ± 1,453; P = 0.7). None of the vaccines induced mean titers consistently higher than those induced by all other vaccines. Response to vaccination as reflected by increase in titer had no effect (P > 0.05) on fecal parvovirus antigen detection.

Discussion

All 3 tests intended for diagnosis of canine parvovirus had strongly positive reactions with feces from a cat with confirmed FPV infection. When these tests were used with feces from recently vaccinated kittens, positive results were occasionally observed, and the frequency of this occurrence varied significantly among the 3 tests. Results were usually weak positives, but 1 kitten had strong positive results with 1 test on 2 of 8 days with positive test results. The reason for the significant differences in the number of positive test results obtained with the 3 different tests is not known, but is likely related to differences in sensitivity (ability of the tests to detect FPV antigens) or specificity (ability of the tests to avoid false-positive results).

A possible explanation for FPV antigen detection in feces after vaccination is replication of live vaccine viruses in lymphoid tissues or intestinal epithelial cells and subsequent shedding of antigens in the feces. However, an unexpected finding was positive antigen test results from 2 of the 3 test brands after administration of inactivated virus vaccines, which do not contain live viruses capable of replication. Although kittens were isolated and barrier procedures were used, it is possible that inadvertent transmission of live vaccine virus occurred, leading to shedding of antigens in kittens in the inactivated virus vaccine group. However, if kittens in the inactivated group were contaminated with MLV vaccine virus, higher serum antibody titers would be expected. The more likely explanation is that false-positive reactions occurred with 2 of the tests, which could have occurred in both the inactivated and MLV vaccine groups.

There was a marked difference in the immune responses of kittens to inactivated versus MLV vaccines at 2 weeks after vaccination. Although both types of vaccines are capable of inducing solid, long-lasting immunity,29,31 MLV vaccines administered parenterally or mucosally are clearly superior to inactivated vaccines when a rapid protective response is required, as is true in shelters.

Results of the present study in SPF kittens may not be completely representative of cats tested in shelters, where higher or lower rates of vaccine interference with parvovirus testing may be observed. The kittens in this study were selected for their negligible FPV antibody titers. This was intended to give MLV vaccines the maximum opportunity for replication and antigen shedding to determine whether vaccination interferes with testing. In contrast, cats in shelters are admitted with a wide range of naturally occurring serum antibody titers that may interfere with vaccine virus replication, which suggests that the frequency of test interference in shelters may be lower than that observed in this study.28 On the other hand, the stress of a sudden change to unfamiliar and crowded surroundings,32-34 possible preexisting debilitating conditions, and concurrent infections may suppress immune function and allow for enhanced replication of the modified-live (attenuated) virus vaccines.35-43 Germfree cats have significantly lower rates of clinical disease and microscopic intestinal lesions after experimental FPV infection than SPF cats do.13,16 This is believed to be a result of a slower rate of epithelial cell division in the unstimulated sterile intestines of germfree cats than the rate in the bacteria-colonized intestines of conventional SPF cats. Because FPV preferentially replicates in rapidly dividing cells, concurrent enteric infectious diseases commonly found in cats in shelters may provide increased capacity for FPV virus replication.30 In this situation, cats in shelters may have higher rates of test interference than the SPF kittens of the present report.

Vaccination of healthy kittens against FPV was associated with positive fecal parvovirus antigen test results, and the frequency of this vaccine interference varied among the 3 tests that were studied. Whenever possible, shelter veterinarians should select vaccines and tests that minimize diagnostic interference after vaccination, in accordance with the principle of differentiating infected from vaccinated animals.44 In addition, positive test results should be interpreted in the context of compatible clinical signs, and confirmatory diagnostic tests should be considered when testing recently vaccinated cats. Although test interference is likely to complicate the identification and control of FPV in shelters, the rate of interference with some tests appears to be low, and most positive test results are subjectively interpreted as weak positives. Despite test interference, the benefit provided by MLV FPV vaccination of all cats upon admission to high-risk environments such as shelters outweighs the negative impact of vaccination on diagnostic test accuracy.

References

- SNAP FIV antibody/FeLV antigen combo test, IDEXX Laboratories, Westbrook, Me.
- FVR C-P, Schering-Plough Animal Health, Omaha, Neb.
- Fel-O-Guard Plus 3, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.
- Eclipse 3, Schering-Plough Animal Health, Omaha, Neb.
- Protex-3, Intervet Inc, Milshoro, Del.
- PureVax Feline 3, Merial Inc, Athens, Ga.
- Feline UltraNasal FVRCP Vaccine, Heska Corp, Fort Collins, Colo.
- Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, NY.
- SNAP Parvo, IDEXX Laboratories, Westbrook, Me.
- AGEN CPV, AGEN Biomedical Ltd, Brisbane, QLD, Australia.
- WITNESS CPV, Synbiotics Corp, San Diego, Calif.
References

1. Associated Press. Virus kills hundreds of cats; keeping pets inside can protect them. Detroit Free Press 2004;August 9:3B.