

Prevalence of feline herpesvirus 1, *Chlamydomphila felis*, and *Mycoplasma* spp DNA in conjunctival cells collected from cats with and without conjunctivitis

Heather C. Low, DVM, MS; Cynthia C. Powell, DVM, MS; Julia K. Veir, DVM, PhD; Jennifer R. Hawley, BS; Michael R. Lappin, DVM, PhD

Objective—To use PCR assays to determine the prevalence of feline herpesvirus 1 (FHV-1), *Chlamydomphila felis*, and *Mycoplasma* spp DNA in conjunctival cells collected from cats with and without conjunctivitis; to compare results of conventional and real-time fluorogenic PCR assays for amplification of FHV-1 DNA; and to determine whether copy numbers of FHV-1 DNA are correlated with conjunctivitis.

Animals—55 cats with active conjunctivitis, 39 healthy cats that never had conjunctivitis, and 32 cats with a history of conjunctivitis that had been resolved for at least 3 months.

Procedures—Samples were obtained by rolling cotton-tipped applicators on the ventral conjunctiva of awake cats treated topically with proparacaine. The DNA was extracted from the swab specimens and assessed in PCR assays to detect DNA of FHV-1 (fluorogenic PCR assay and conventional PCR assay), *Mycoplasma* spp (conventional PCR assay), and *C felis* (conventional PCR assay).

Results—Overall prevalence rates of FHV-1, *C felis*, and *Mycoplasma* spp as assessed by the conventional PCR assays were 6.7%, 3.2%, and 9.6%, respectively. Percentage concordance between conventional PCR and fluorogenic PCR assays for FHV-1 was 92.5%. There were no significant differences among the 3 groups of cats for the mean copy number of FHV-1 divided by the copy number of glyceraldehyde-3-phosphate dehydrogenase.

Conclusions and Clinical Relevance—*Mycoplasma* spp were the most prevalent organism detected and was associated with conjunctivitis. This study could not confirm that there are increased copy numbers of FHV-1 DNA in cats with conjunctivitis, compared with the copy numbers for cats without conjunctivitis. (*Am J Vet Res* 2007;68:643–648)

Feline herpesvirus 1 is one of the most common infectious disease agents of cats worldwide and is believed to be the most common cause of ocular disease in cats. A member of the alpha herpesvirus subfamily, FHV-1 is highly species specific and can be transmitted among cats via direct contact with infected ocular and respiratory secretions. *Chlamydomphila felis* and *Mycoplasma* spp are also associated with ocular disease in cats, in particular conjunctivitis, and clinical disease with these organisms is believed to be potentiated by concurrent FHV-1 infection.¹⁻³

Feline herpesvirus 1 replicates in the mucous membranes of the respiratory tract, conjunctiva, and cornea and is frequently associated with clinical disease, especially in primary infections in kittens. Latency, with the virus residing principally in the trigeminal ganglion, will develop after primary infection in approximately 80% of infected cats.⁴⁻⁶ Approximately half of latently

ABBREVIATIONS	
FHV-1	Feline herpesvirus 1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Ct	Threshold cycle
Con+	Positive results on the conventional PCR assay
FG+	Positive results on the fluorogenic PCR assay
Con-	Negative results on the conventional PCR assay
FG-	Negative results on the fluorogenic PCR assay

infected cats will have spontaneous and stress-related reactivation and shedding of the virus, and although some cats will develop associated clinical disease (primarily conjunctivitis or keratitis), many cats will not.⁷

Ocular disease from recrudescence FHV-1 infection can be quite severe. Although effective control of clinical signs can be achieved in most affected cats through the use of antiviral drugs, these drugs have been developed for use against human herpes simplex virus and are less than ideal for use against FHV-1 in cats. For example, the systemic antiviral drug valacyclovir can cause bone marrow suppression, renal necrosis, and liver necrosis in cats.⁸ Acyclovir, another systemic

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From the Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523. Dr. Low's present address is WestVet Emergency and Specialty Center, 5019 N Sawyer Ave, Garden City, ID 83714.

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Address correspondence to Dr. Powell.

antiviral drug, does not reach therapeutic plasma concentrations in cats and has limited effectiveness against FHV-1 because of differences in virus-encoded thymidine kinase.^{9,10} Topical antiviral drugs are expensive, often irritating, and require application 4 to 6 times/d to be effective.

Because of these treatment limitations, there is substantial need for a diagnostic test that can accurately differentiate cats with ocular disease caused by FHV-1 from cats with other ocular disorders. Antibodies against FHV-1 can be detected by serum neutralization tests or ELISAs, but positive results do not differentiate natural exposure from vaccination, do not correlate with current infection, and do not correlate with disease status.¹¹ Current FHV-1 infection can be determined by virus isolation, immunofluorescence techniques, and conventional PCR assays, but positive test results do not differentiate vaccine strains from natural strains and do not differentiate subclinically infected cats from those with clinical disease as a result of FHV-1 infection.^{11,12} In fact, in 1 study¹³ in which investigators used a conventional PCR assay, FHV-1 DNA was detected more commonly in cats without conjunctivitis than in cats with conjunctivitis.

Fluorogenic PCR assays can be used to quantify the amount of DNA contained in a sample by comparing the target DNA copy numbers to those of a housekeeping gene that is consistently found in all mammalian cells. By quantifying the DNA, test results may be more meaningful than those for conventional PCR assays in which results are reported only as positive or negative. In 1 study¹⁴ in which a fluorogenic PCR assay was used to determine FHV-1 DNA copy numbers in ocular fluid of cats with conjunctivitis, it was revealed that results for the fluorogenic PCR assay may correlate with clinical disease. However, only a small number of cats were assessed in that study, and results were not compared with naturally exposed cats without clinical disease. When a similar fluorogenic PCR assay was applied to samples collected from cats with and without clinical evidence of rhinitis, DNA copy numbers correlated with disease status.^a

The objectives of the study reported here were to use PCR assays to determine the prevalence of FHV-1, *C felis*, and *Mycoplasma* spp DNA in conjunctival cells collected from cats with and without conjunctivitis; to compare results of end point and real-time fluorogenic PCR assays for amplification of FHV-1 DNA; and to determine whether copy numbers of FHV-1 DNA measured by use of fluorogenic PCR assays can be correlated with disease status.

Materials and Methods

Animals—Three groups that comprised 126 client-owned cats were included in the study. Samples were collected from 39 healthy cats that never had conjunctivitis, 32 cats with a history of conjunctivitis that had been resolved for at least 3 months, and 55 cats with active conjunctivitis. This study was approved by the Colorado State University Animal Care and Use Committee. All owners were informed that samples from their cats would be tested for *C felis*, *Mycoplasma* spp, and FHV-1, and written consent was obtained for ex-

amination and diagnostic testing when entered into the study.

To prospectively determine the prevalence of selected infectious agents in cats with and without conjunctivitis, an e-mail that detailed the study and called for sample submissions was sent to Colorado State University faculty, staff, and students; members of the American College of Veterinary Ophthalmologists; and members of the Larimer County Veterinary Medical Association. Interested persons were then sent instructions for sample collection and shipment. Cats were excluded when vaccines that contained modified-live FHV-1 had been administered within the preceding month or cats were currently being treated topically or systemically with antiviral drugs. Cats treated systemically with antimicrobials or lysine or topically with other ophthalmic medications were included.

Sample collection—Samples were obtained by gently rolling dry cotton-tipped applicators on the ventral conjunctiva of awake, nonsedated cats whose eyes had been treated topically with proparacaine. Swab specimens were then placed in 1.0 mL of sterile 0.01M PBS solution and allowed to equilibrate at 22°C for 2 to 3 hours. Samples from cats that resided outside Larimer County, Colo, were collected and processed in a similar manner, frozen at -20°C, and then placed on ice and shipped to the Colorado State University Infectious Disease Laboratory by overnight express mail. All samples were stored at -70°C until analyzed. Samples were thawed and allowed to equilibrate at 22°C for 2 to 3 hours before assay.

DNA assays—The DNA was extracted from samples by use of a commercially available kit^b in accordance with the manufacturer's protocols for swab specimens. Fifty micrograms of salmon sperm DNA^c was added to each milliliter of manufacturer's lysis buffer. Briefly, the swab was pressed against the side of the tube to expel all possible liquid; the swab was then discarded. The sample was vortexed, transferred to a microcentrifuge tube that contained 1.5 mL of RNase- and DNase-free solution, and centrifuged at 500 × g for 5 minutes. Supernatant was decanted, and the pellet was resuspended in 400 μL of sterile 0.01M PBS solution. Samples were assayed for FHV-1 by use of a conventional PCR assay¹³ and a real-time PCR assay,¹⁴ *C felis* by use of a conventional PCR assay,¹⁵ *Mycoplasma* spp by use of a conventional PCR assay,¹⁶ and GAPDH by use of a real-time PCR assay.

Quantitation of FHV-1 DNA by use of a real-time PCR assay was adapted from a protocol published elsewhere.¹⁴ Briefly, the 25-μL real-time PCR reaction contained 12.5 μL of a commercial mastermix^d containing 100mM KCl, 40mM Tris-HCl, 1.6mM dNTPs, 6mM MgCl₂, and 50 U of DNA polymerase/mL; 0.5 μL (400nM) of each primer; 0.2 μL (80nM) of probe^e; 1.3 μL of sterile water; and 10 μL of extracted template DNA. All reactions were performed on a thermocycler^f for the following conditions: 2 minutes at 50°C, 10 minutes at 95°C, and then 40 cycles (each cycle consisted of a denaturation step [15 seconds at 95°C] followed by an annealing step [1 minute at 60°C]). Parallel reactions for feline GAPDH in accordance with another proto-

col¹⁷ were performed on the same plate to account for variations in DNA quantity in each sample secondary to differences in yields of cells during collection of swab specimens. The GAPDH reaction conditions were the same as those for FHV-1, except that only 5 µL of template DNA was added with a corresponding increase in the volume of sterile water (6.3 µL).

Data were analyzed by use of the instrument software. Samples were considered to have positive results when the fluorescence intensity exceeded 10 times the SD of the baseline fluorescence. The cycle at which each sample signal crossed this threshold was considered to be the Ct for that sample. All reactions were conducted in triplicate. A control sample consisting of DNA pooled from swab specimens obtained from 3 cats that previously had strong positive reactions for both FHV-1 and GAPDH DNA was included on each plate to verify repeatable thermocycler conditions.

A standard curve for GAPDH cell equivalent was generated by use of RNA isolated from a feline lung epithelial cell line developed by our laboratory group that was digested in the same manner as the test samples. The standard curve for FHV-1 was generated by a series of 10-fold dilutions by use of plasmid-generated DNA. The FHV-1 plasmid-generated DNA was created by use of a commercially available vector^a after purification^b of product obtained from a conventional PCR reaction by use of the primers used in the real-time assay. All samples for the standard curve were assayed in duplicate. Mean FHV-1 DNA copy number was divided by the mean GAPDH copy number to obtain the cell equivalent for each sample.

To determine whether tears contain factors that could inhibit FHV-1 PCR assays, tears from an FHV-1-negative, healthy, specific-pathogen-free cat were collected on a Schirmer tear test strip (up to 25 mm). On a second (control) Schirmer tear test strip, a corresponding amount of PBS solution was added (up to 25 mm). The strips were placed in identical 1.5-mL centrifuge tubes, 50 µL of PBS solution was added to each tube, and the tubes were centrifuged at 10,000 × g for 5 minutes. Supernatants were used to make 5 serial logarithmic dilutions of genomic FHV-1 DNA; the dilutions were assayed by use of the fluorogenic FHV-1 PCR assay.

Data analysis—Prevalence rates for DNA of FHV-1, *C felis*, and *Mycoplasma* spp were defined on the basis of the 3 conventional PCR assays. Prevalence rates for the various infectious agents, alone or in combination, were compared among the 3 groups of cats with and without conjunctivitis by use of the 2-tailed Fisher exact probability test. Because it is unknown whether the conventional or fluorogenic FHV-1 PCR assay is the criterion-referenced standard, sensitivity and specificity could not be calculated. Thus, results of the conventional and fluorogenic PCR assays were compared by calculating percentage concordance by use of the following equation:

$$\frac{(\{Con+ \text{ and } FG+\} + \{Con- \text{ and } FG-\})}{\{Con+ \text{ and } FG+\} + \{Con- \text{ and } FG-\} + \{Con+ \text{ and } FG-\} + \{Con- \text{ and } FG+\}} \times 100$$

Finally, to determine whether FHV-1 copy numbers as determined by the fluorogenic PCR assay correlated

with disease status (ie, conjunctivitis), mean results for each group of cats were calculated and compared by use of the unpaired Student *t* test. Results for each group of cats were compared, and results for the healthy cats were then compared with the combined results for cats with a history of conjunctivitis that had been resolved for at least 3 months and with results for cats with active conjunctivitis. For all statistical comparisons, significance was defined at values of *P* < 0.05.

Results

Prevalence—All assays (FHV-1, *C felis*, and *Mycoplasma* spp) were not performed on every sample because there was insufficient sample volume resulting from the need to repeat several fluorogenic PCR assays as a result of equipment difficulties. Thus, conventional PCR assay for FHV-1 was performed on 120 samples (39 healthy cats that never had conjunctivitis, 32 cats with a history of conjunctivitis that had been resolved for at least 3 months, and 49 cats with active conjunctivitis). Conventional PCR assay for *C felis* was performed on 124 samples (37 healthy cats, 32 cats with a history of conjunctivitis, and 55 cats with active conjunctivitis). Conventional PCR assay for *Mycoplasma* spp was performed on 125 samples (39 healthy cats, 31 cats with a history of conjunctivitis, and 55 cats with active conjunctivitis). The overall prevalence rates of FHV-1, *C felis*, and *Mycoplasma* spp DNA as determined by conventional PCR assays were 6.7%, 3.2%, and 9.6%, respectively (Table 1). When prevalence rates were compared among groups of cats, cats with active conjunctivitis were significantly (*P* = 0.042) more likely to have *Mycoplasma* spp DNA amplified from conjunctival swab specimens, compared with results for healthy cats. Although FHV-1 and *C felis* DNA were amplified more often from conjunctival swab specimens of cats with active conjunctivitis than from those of healthy cats, the differences between these groups were not significant. However, *C felis* DNA was amplified only from cats with active conjunctivitis.

All 3 assays were performed on 116 samples. Of these, DNA of an infectious agent was amplified from 35.3%, 10.0%, and 5.7% of the cats with active con-

Table 1—Prevalence of FHV-1, *Chlamydomphila felis*, and *Mycoplasma* spp DNA as determined by conventional PCR assays performed on conjunctival swabs specimens collected from client-owned cats.

Animals	FHV-1	<i>C felis</i>	<i>Mycoplasma</i> spp
	No. (%)	No. (%)	No. (%)
Healthy cats	1/39 (2.6)	0/37 (0)	1/39 (2.6)
Cats with conjunctivitis			
History of conjunctivitis	1/32 (3.1)	0/32 (0)	2/31 (6.5)
Active conjunctivitis	6/49 (12.2)	4/55 (7.3)	9/55 (16.4)*
Combined history and active	7/81 (8.6)	4/87 (4.6)	11/86 (12.8)
Total	8/120 (6.7)	4/124 (3.2)	12/125 9.6

Values reported represent the number of samples with positive results/number of samples tested. All 3 assays were not performed on every sample.
*Within a column, prevalence differs significantly (*P* < 0.05; 2-tailed Fisher exact test) from the prevalence for the healthy cats.

conjunctivitis, cats with a history of conjunctivitis that had been resolved for at least 3 months, and healthy cats that never had conjunctivitis, respectively. When prevalence rates were compared among the groups of cats for the 116 samples on which all 3 assays were performed, cats with active conjunctivitis ($P = 0.001$) and the combination of cats with active conjunctivitis and cats with a history of conjunctivitis that had been resolved for at least 3 months ($P = 0.008$) were significantly more likely to have DNA of an infectious agent amplified from conjunctival swab specimens, compared with results for healthy cats that had never had conjunctivitis.

Multiple infections were uncommon. For example, none of the 116 samples tested for all 3 organisms had positive results for DNA of both FHV-1 and *C felis*. Two samples had positive results for DNA of both *Mycoplasma* spp and *C felis*; both were from cats that had active conjunctivitis. One sample had positive results for DNA of both *Mycoplasma* spp and FHV-1; that sample was also obtained from a cat that had active conjunctivitis.

At the time of sample submission, it was known that 21 cats were currently being administered L-lysine; of these, 14 had active conjunctivitis. Only 1 cat with active conjunctivitis that was treated with L-lysine had positive results for FHV-1 by use of the fluorogenic PCR assay, and none had positive results by use of the conventional PCR assay.

Comparison of conventional PCR assay with fluorogenic PCR assay—The distribution of results of the conventional PCR assay and fluorogenic PCR assay was determined for 120 samples obtained from client-owned cats with and without conjunctivitis for which both assays were performed. Percentage concordance between the 2 assays was 92.5%. Both assays yielded positive results for 5 samples and negative results for 106 samples. Divergent results were obtained for 9 samples (Con+ and FG– on 3 samples and Con– and FG+ on 6 samples). Of the 6 samples that were FG+ but Con–, 2 were from healthy cats, 2 were from cats with a history of conjunctivitis, and 2 were from cats with active conjunctivitis (Table 2). Of the 3 samples that were FG– but Con+, 1 was from a healthy cat and 2 were from cats with active conjunctivitis.

Correlation of FHV-1 copy number to disease status—We did not obtain differences in FHV-1 detection sensitivity, as determined by use of the fluorogenic PCR assay, when results between FHV-1–containing samples diluted in tears and FHV-1–containing samples diluted in PBS solution were compared (data not shown).

Of the 120 samples on which fluorogenic PCR assay was performed, 11 (2 healthy cats, 3 cats with a history of conjunctivitis that had been resolved for at least 3 months, and 6 cats with active conjunctivitis) had positive results when tested for FHV-1 DNA by use of conventional PCR assay (Table 2). Group mean \pm SD of the FHV-1 copy number divided by the GAPDH copy number for healthy cats, healthy cats with a history of conjunctivitis that had been resolved for at least 3 months, and cats with active conjunctivitis was 0.0027 ± 0.010 , 0.0006 ± 0.002 , and 0.0197 ± 0.130 , respectively. There were no significant differences in these values among the 3 groups of cats.

Table 2—Distribution of results for fluorogenic PCR and conventional PCR assays for amplification of FHV-1 DNA in samples obtained from cats with and without conjunctivitis.

Animals	Fluorogenic PCR	Conventional PCR
Healthy cats	Positive (0.0410)	Negative
	Positive (0.0632)	Negative
	Negative (—)	Positive
History of conjunctivitis	Positive (0.0049)	Negative
	Positive (0.0072)	Negative
	Positive (0.0069)	Positive
Active conjunctivitis	Positive (0.0006)	Negative
	Positive (0.8920)	Negative
	Positive (0.0024)	Positive
	Positive (0.0108)	Positive
	Positive (0.0122)	Positive
	Positive (0.0454)	Positive
	Negative (—)	Positive
	Negative (—)	Positive

Values in parentheses represent the FHV-1 copy number divided by the GAPDH copy number.
— = Not applicable.

Discussion

The study reported here was conducted to determine the prevalence of FHV-1, *C felis*, and *Mycoplasma* spp DNA for 120, 124, and 125 cats, respectively. *Mycoplasma* sp was the most prevalent organism detected (9.6%), followed by FHV-1 (6.7%) and *C felis* (3.2%). The DNA of an infectious agent was detected in significantly more cats with conjunctivitis than in healthy cats, suggesting that the organisms played a role in the pathogenesis of disease in at least some of the cats.

Detection of *Mycoplasma* spp DNA in significantly more cats with active conjunctivitis than in healthy cats suggests that the organism can be associated with disease. In other studies,^{18–20} pathogenicity of *Mycoplasma* sp has been questioned because it is frequently detected in samples obtained from the conjunctiva of clinically normal cats and disease is difficult to reproduce experimentally. Although it has been suggested^{3,21} that *Mycoplasma* sp is pathogenic only when it is associated with other organisms, such as FHV-1, we found in the study reported here that only 1 of 116 cats had positive results for both *Mycoplasma* spp and FHV-1 DNA. Although FHV-1 and *C felis* DNA were amplified more often from conjunctival swab specimens of cats with active conjunctivitis than from those of healthy cats, the differences between groups were not significant. However, *C felis* DNA was amplified only from cats with active conjunctivitis.

Specific information on antimicrobial treatment of cats was not available. It is possible that cats of our study were being treated with antimicrobials, such as doxycycline, that could have reduced the detection rates of *C felis* and *Mycoplasma* spp in cats with conjunctivitis. However, it is unlikely the healthy cats were being treated with drugs that would have falsely lowered the prevalence rates for those organisms. In future prevalence studies, it should be verified that samples are collected before administration of antimicrobial treatments.

The overall detection rate for FHV-1 DNA in our study was determined to be 9.2% by use of a fluoro-

genic PCR assay and 6.7% by use of a conventional PCR assay, whereas the detection rate for cats with conjunctivitis was 12.2% for both fluorogenic and conventional PCR assays. In other studies,^{1,2,11-13,22-26} detection rates of FHV-1 for various detection methods, including several types of PCR assays, virus isolation, and fluorescent antibody testing, ranged from 1% to 88.9%. This wide variation is attributable to the fact that detection rates vary with the disease state, population tested, type of sample tested, and method of detection.

The low detection rate of FHV-1 DNA in cats with active conjunctivitis in the study reported here was unexpected, and there are several explanations for this finding. As mentioned previously, *C felis* and *Mycoplasma* spp could have been the cause of conjunctivitis in some of the cats. However, there were many other cats with conjunctivitis in the study from which we were not able to amplify DNA of an infectious agent associated with conjunctivitis. It is possible that these cats had another unrecognized cause of conjunctivitis, such as calicivirus.

Alternately, FHV-1 could still have played a role in the disease process but was not in detectable amounts in conjunctival swab specimens. The inclusion of cats being administered L-lysine could have decreased the FHV-1 detection rate by reducing viral shedding.^{27,28} To assess this hypothesis, samples should be collected before administration of L-lysine in future studies.

Sample collection methods may also affect FHV-1 detection rates. In the study reported here, samples were collected by swabbing the conjunctiva with a sterile cotton-tipped applicator. Although DNA was detected in all of the samples collected by use of a conjunctival swab (positive results for GAPDH in all samples), it is possible that there was not a sufficient sample of representative cells containing FHV-1 DNA in the swab specimens. Investigators in other studies²³⁻²⁵ have collected samples with a cytobrush or via conjunctival biopsy. It would seem reasonable that a larger, deeper tissue sample with an increased number of cells for testing (eg, biopsy specimen) would be associated with an increased detection rate.²⁹ This may be particularly important when attempting to diagnose chronic or recrudescing infections when viral shedding is decreased, especially because FHV-1 is an obligate intracellular virus. However, it was reported in 1 study²⁵ that there was no difference in the ability to harvest detectable FHV-1 DNA whether the samples were collected by use of swabs, scrapings, or biopsies, except for samples from corneal sequestra. In addition, it was not specified in our study whether samples were collected from 1 or both eyes. In cats with obvious clinical signs of conjunctivitis, swabbing the affected eye would be logical. However, FHV-1 reactivation can be mild and unilateral. Thus, it would have been prudent to collect samples bilaterally in cats without obvious clinical signs because swabbing the unaffected eye would likely yield a false-negative result.

The prevalence of FHV-1 may also differ with the population tested. In the study reported here, samples were collected from client-owned animals with vary-

ing disease states and clinical signs. In another study¹³ in which samples were collected from shelter cats, the prevalence was 31%. It is plausible that the increased rate of detection in that study was attributable to the highly infectious nature of FHV-1, which allowed it to be easily spread throughout a population (ie, shelter cats) that is in close contact with each other at a single facility.

A further limitation of our study was that classification was frequently based on the recollections of owners, which may not have been accurate. Many client-owned animals are adopted as adults from various shelters and humane societies. It is possible that many of these animals had episodes of conjunctivitis as kittens that were not observed by their current owners. This may also explain how it was possible to obtain positive results for PCR assays on samples obtained from healthy cats that supposedly never had an episode of conjunctivitis. However, that finding may also be explained by the persistence of modified-live FHV-1 vaccine strains or subclinical carriage of the organism.

The effect of shipping temperature on the ability to detect FHV-1 DNA was also considered. It was requested that samples submitted by collaborators from outside the Colorado State University veterinary teaching hospital be frozen and shipped on ice, but many samples arrived at the laboratory with melted ice and at ambient temperature. However, in another study,³⁰ results of PCR assays did not differ for identical samples sent overnight on ice or mailed at ambient daytime temperatures during a period of several days. This indicates that shipping temperature may not have played a large role in the detection of FHV-1 DNA in our study.

Percentage concordance between the 2 FHV-1 PCR assays was 92.5%. There were several samples that were discordant (Table 2). Because appropriate positive and negative control samples were included with each assay, it is unlikely the discordant results were caused by laboratory error. For 5 of the 6 samples that had positive results for the fluorogenic PCR assay but negative results for the conventional PCR assay, the ratio of FHV-1 copy number to GAPDH copy number was relatively low. Although the 2 PCR assays were conducted in parallel, it is possible that in samples with low FHV-1 DNA copy numbers, the small volumes of sample digest used for PCR amplification could have contained varying amounts of DNA, which may have led to discordant results between the 2 assays. One of the samples that had positive results for the fluorogenic PCR assay but negative results for the conventional PCR assay had a relatively high ratio of FHV-1 copy number to GAPDH copy number; this sample was likely a false-negative result in the conventional PCR assay.

Although cats with active conjunctivitis in the study reported here had higher FHV-1 DNA copy numbers than were determined for the other 2 groups of cats, the difference in copy numbers was not significant. In contrast, investigators in another study¹⁴ used fluorogenic PCR assays to determine FHV-1 DNA copy numbers in ocular fluid of cats with conjunctivitis. They found that results may correlate with clinical disease, such that consecutive samples would allow tracking of the course of infection. Our failure to find an as-

sociation may simply have been related to the number of samples. We had only 11 of 120 samples with positive results, and a greater number of samples should be assessed to discern whether the fluorogenic PCR assay will be a valuable tool for determining whether cats with conjunctivitis attributable to infection with FHV-1 have a higher DNA copy number than unaffected cats. However, because ocular disease associated with FHV-1 infection is believed to be, in part, a hypersensitivity reaction against the virus,³¹⁻³³ it is possible that by the time samples are obtained from clinically affected cats, FHV-1 DNA copy numbers are below the detectable limits of the assay, even though the inflammation is still associated with the virus.

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