

# Development of a reverse transcriptase-polymerase chain reaction assay to detect feline herpesvirus-1 latency-associated transcripts in the trigeminal ganglia and corneas of cats that did not have clinical signs of ocular disease

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**Objective**—To develop a reverse transcriptase-polymerase chain reaction (RT-PCR) assay to detect feline herpesvirus-1 (FHV-1) latency-associated transcripts (LATs) in the corneas and trigeminal ganglia of cats that did not have clinical signs of ocular disease.

**Sample Population**—Corneas and trigeminal ganglia obtained from 21 cats necropsied at the Indiana Animal Disease Diagnostic Laboratory and 25 cats euthanatized at a humane shelter; none of the cats had a recent history of respiratory tract or ocular disease, and all had normal results for ophthalmic examinations.

**Procedure**—Both corneas and both trigeminal ganglia were harvested from each cat. An initial PCR assay detected FHV-1 DNA in the corneas and trigeminal ganglia. The RNA was then isolated from samples positive for FHV-1 DNA, and an RT-PCR assay was used to detect LATs.

**Results**—FHV-1 DNA was detected in 45 of 92 (48.9%) corneas and 38 of 92 (41.3%) trigeminal ganglia. In many samples, the RNA had degraded and RT-PCR assay was not possible. Of the samples subjected to RT-PCR assay, none of the 39 corneas but 4 of 16 trigeminal ganglia had positive results when tested for LATs.

**Conclusions and Clinical Relevance**—Analysis of the results indicated that a high percentage of cats that did not have clinical signs of ocular disease had detectable FHV-1 DNA in their corneas and trigeminal ganglia. This study documents that the RT-PCR assay can successfully identify LATs and may serve as a tool to better understand the biologic characteristics of FHV-1 and its relationship to clinical disease. (*Am J Vet Res* 2004;65:314–319)

tion is a major cause of respiratory tract and ocular infections in cats<sup>1</sup> and establishes latent infections with an estimated incidence of 80%.<sup>2</sup> Feline herpesviral DNA has been detected by use of a **polymerase chain reaction (PCR)** assay in 5.9% to 46% of corneas from cats without clinical signs of ocular disease.<sup>3,4</sup> Feline herpesviral DNA has also been detected by use of a PCR assay in 18% to 55% of corneal sequestra in cats and 76.3% of corneal scrapings from cats with eosinophilic keratitis.<sup>3,4</sup> However, although FHV-1 DNA has been detected, the replicative status of the virus has not been determined. The detection of FHV-1 DNA may represent active viral infection, latent virus, or DNA remnants from degraded virus particles.

The ability to establish neuronal latency is a hallmark of the Alphaherpesvirinae.<sup>5</sup> The classic definition of latency is a period without clinical signs of disease during which infectious virus cannot be recovered from fresh homogenized tissues but virus can be induced to reactivate by various stimuli, such as explantation and maintenance in organ culture.<sup>6,7</sup> Originally, it was believed that viral transcription did not occur during latency. However, viral transcripts of small strands of RNA, termed **latency-associated transcripts (LATs)**, have been detected in latently infected neurons.<sup>5</sup> Although the function and importance of LATs are not known, they provide a molecular marker for latently infected cells<sup>5,8</sup> and a tool to distinguish low-level persistent infection from true latency.<sup>9</sup> In 1 study,<sup>10</sup> investigators detected LATs in the trigeminal ganglia of experimentally infected cats by use of in situ hybridization and northern blot analysis. However, LAT expression is extremely low in a great proportion of latently infected neurons.<sup>11</sup> In another study,<sup>11</sup> investigators documented that the number of LAT-positive cells detected by use of an in situ reverse transcriptase (RT)-PCR assay was substantially greater than the number of cells identified as LAT-positive by use of in situ hybridization. Therefore, the study reported here was conducted to develop an RT-PCR assay to detect FHV-1 LATs and evaluate use of the RT-PCR assay on corneas and trigeminal ganglia obtained from cats that did not have clinical signs of ocular disease.

## Materials and Methods

**Sample population**—Tissue specimens were obtained from 2 groups of cats for use in the study. Group 1 consisted of 25 cats euthanatized by a local humane shelter, com-

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prising 18 domestic shorthair and 7 domestic longhair cats (11 sexually intact males, 2 neutered males, and 12 females). The cats ranged from approximately 3 months to 10 years of age (mean  $\pm$  SD,  $2.71 \pm 2.65$  years). Group 2 consisted of 21 cats necropsied at the Indiana Animal Disease Diagnostic Laboratory, comprising 16 domestic shorthair cats, 1 domestic longhair cat, 2 Maine Coons, 1 Siamese, and 1 Persian (2 sexually intact males, 10 neutered males, 3 sexually intact females, and 6 spayed females). The cats ranged from 8 weeks to 20 years of age (mean,  $7.52 \pm 6.10$  years). The medical history revealed that all cats did not have recent respiratory tract or ocular lesions. All cats were examined by use of slit-lamp biomicroscopy and were excluded from the study when lesions were detected in the anterior ocular segment.

**Sample collection**—Sterile technique was used to collect specimens of both corneas and both trigeminal ganglia from all cats; these specimens were used for PCR and RT-PCR assays. We collected a central portion of each cornea (approx  $8 \times 8$  mm), which was divided into 2 equal parts. Half of each corneal specimen was placed in 1 mL of PBS solution and stored at  $-70^\circ\text{C}$  for subsequent DNA extraction. The other half was placed in 1 mL of RNA preservation media<sup>4</sup> and stored overnight at  $4^\circ\text{C}$ . This sample was then transferred to a sterile microcentrifuge tube and stored at  $-70^\circ\text{C}$  for subsequent RNA extraction. Each trigeminal ganglion was collected in its entirety, sectioned into 2 equal parts, and stored in the same manner as described for the corneas.

**Control samples of viral DNA and RNA**—A field isolate of FHV-1 propagated in cultured Crandell feline kidney cells was used as a positive-control sample. Viral infectivity assessed by use of quantal titration was  $1.84 \times 10^8$  TCID<sub>50</sub>/mL. A mixture of 5  $\mu\text{L}$  of virus-containing solution and 20  $\mu\text{L}$  of K-buffer<sup>4</sup> containing 2  $\mu\text{g}$  of proteinase K/ $\mu\text{L}$  was heated at  $56^\circ\text{C}$  for 2 hours. Proteinase K was then inactivated by heating at  $94^\circ\text{C}$  for 10 minutes. The DNA was purified by use of standard phenol-chloroform extraction and ethanol precipitation.<sup>12</sup> The RNA was extracted from 1 mL of virus-containing solution by use of a phenol-guanidine thiocyanate protocol<sup>b</sup> and quantified via spectrophotometry.

**Assay of feline DNA**—To ensure that DNA had been extracted from each feline tissue sample, published primers were used to amplify a 290-bp segment of the feline *c-fes* proto-oncogene.<sup>15</sup> The 100- $\mu\text{L}$  reaction mixture consisted of 10  $\mu\text{L}$  of extracted DNA, 50mM KCl, 10mM Tris-HCl (pH, 9.0 at  $25^\circ\text{C}$ ), 1.5mM MgCl<sub>2</sub>, 0.1% Triton X-100, 5 units of *Taq* polymerase,<sup>c</sup> 250 $\mu\text{M}$  of each deoxynucleoside triphosphate (dNTP), and 0.2 $\mu\text{M}$  of each oligonucleotide primer. Amplification was performed in an automated thermocycler<sup>d</sup> in accordance with a published protocol.<sup>13</sup> Amplification products were electrophoresed on a 1.5% agarose gel containing ethidium bromide and then photographed under ultraviolet illumination. Only those samples that had positive results for feline DNA were subsequently evaluated for FHV-1 DNA.

**Assay of feline RNA**—To ensure the quantity and quality of the RNA extracted from each feline tissue sample, all samples were subjected to an RT-PCR assay by use of published primers that amplify an 82-bp portion of the feline glyceraldehyde-3-phosphate dehydrogenase RNA transcript.<sup>14</sup> Modifications to the published protocol<sup>e</sup> included use of 0.9 $\mu\text{M}$  of each primer; 0.5  $\mu\text{g}$  of sample RNA; and 40 cycles of 30 seconds at  $94^\circ\text{C}$ , 30 seconds at  $65^\circ\text{C}$ , and 1 minute at  $68^\circ\text{C}$ . To ensure that each sample was free of

contaminating DNA, the same PCR assay was performed by use of 5 units of *Taq* polymerase<sup>c</sup> in place of the RT *Taq* polymerase enzyme mix.<sup>f</sup> When there was no contaminating DNA and *Taq* polymerase<sup>c</sup> was used in place of the RT *Taq* polymerase enzyme mix,<sup>f</sup> there were no PCR products. All amplification products were electrophoresed on a 1.5% agarose gel stained with ethidium bromide and photographed under ultraviolet illumination. Positive- and negative-control samples were included with each RT-PCR assay.

**DNA extraction**—Corneal samples (25  $\mu\text{g}$ ) or trigeminal ganglia samples (6  $\mu\text{g}$ ) were placed in 400  $\mu\text{L}$  of K-buffer<sup>f</sup> containing 0.1  $\mu\text{g}$  of proteinase K/ $\mu\text{L}$ . Samples were incubated overnight at  $60^\circ\text{C}$ . Proteinase K was then inactivated by heating at  $94^\circ\text{C}$  for 10 minutes. The DNA was purified by use of phenol-chloroform extraction and ethanol precipitation. The pellet was suspended in 20  $\mu\text{L}$  of Tris-EDTA buffer (10mM Tris and 1mM EDTA [pH, 8.0]).

**PCR amplification of FHV-1 DNA**—Published primers were used in accordance with published protocols to amplify a 224-bp fragment of the FHV-1 thymidine kinase gene.<sup>15</sup> Modifications to the protocol included the use of a single PCR test with the internal oligonucleotide primers, 5 units of *Taq* polymerase,<sup>c</sup> and 0.2 $\mu\text{M}$  of each oligonucleotide primer.

Precautions to prevent contamination included separate areas for handling specimens before and after amplification, 1-time use of filter pipette tips, use of latex gloves, placement of used pipette tips in a waste container with 0.5% NaOH, and frequent cleaning of laboratory surfaces with 0.5% NaOH. Positive-, negative-, and digest-control samples were included in each assay. Feline WBC DNA was used to determine that the FHV-1 primers would not amplify feline DNA. Amplification products were electrophoresed on a 1.5% agarose gel containing ethidium bromide and photographed under ultraviolet illumination. The PCR products were then transferred to nylon membranes.<sup>8</sup> Specificity of the PCR products was confirmed by hybridization to a digoxigenin-labeled 20-mer oligonucleotide probe (FHV-1 probe, 5'-GCC TAT ACC GCC CAC TA-3'), which bound a DNA sequence internal to the PCR primers. Limit of detection of the probe was 30fM/ $\mu\text{L}$ . Additionally, automated DNA sequencing<sup>h</sup> verified the specificity of the amplification product because the sequenced fragment had 99% homology with the expected product. Only those samples confirmed as having positive results after hybridization were included as samples with positive results in the study.

**RNA extraction**—The RNA was extracted from corneal and trigeminal ganglia samples that had positive results when tested for FHV-1 DNA by use of the PCR assay. Tissue samples were placed separately in sterile foil packets, immersed

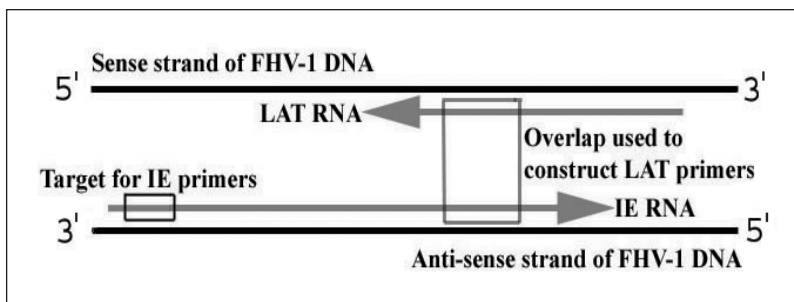


Figure 1—Diagram depicting the feline herpesvirus type-1 (FHV-1) genome and areas of latency-associated transcript (LAT) RNA and transcription of the immediate early (IE) gene to determine regions selected as targets for the LAT and IE oligonucleotide primers used in a reverse transcriptase (RT)-polymerase chain reaction (PCR) assay. The area of overlap in the regions of LAT RNA and IE transcription allowed the use of the IE gene sequence to construct the LAT primers.

in liquid nitrogen, pulverized, and then placed in a vial containing 1 mL of a phenol-guanidine thiocyanate solution.<sup>b</sup> The RNA was extracted in accordance with a published phenol-guanidine thiocyanate protocol<sup>b</sup> and quantified via spectrophotometry.

**RT-PCR oligonucleotide primers**—The FHV-1 LATs are anti-sense to and overlap with the 3' end of the immediate

early (IE) gene,<sup>10</sup> the sequence of which has been determined.<sup>16</sup> Directional primers were constructed to amplify RNA transcribed anti-sense to the 3' end of the IE gene. These primers allowed amplification of the LATs (Fig 1). The sequence of the primer (LAT primer 1) used to synthesize the LAT cDNA was 5'-TGG ATG GTT CCA TAA CAG CA-3', which corresponds to base numbers 5896 to 5915 of the IE gene sequence. The second LAT primer (LAT primer 2) was 5'-CAC AAA CCG CCA GAA CTA CA-3', which is complementary to the IE gene sequence base numbers 6095 to 6076. These primers amplify a 200-bp segment of LAT RNA.

A separate set of directional primers was constructed to amplify RNA transcribed normally from the 5' end of the IE gene. These primers amplified IE RNA, which would allow investigation of early transcriptional activity of the FHV-1 genome. These primers also acted as a control measure to ensure that the directional LAT primers were not amplifying portions of IE RNA. The primer (IE primer 1) used to synthesize IE cDNA was 5'-GGG TCC CGA GGT AAT TGT TT-3', which is complementary to the IE gene sequence base numbers 2068 to 2049. The second IE primer (IE primer 2) was 5'-TGT AGA GTC GAG GGG ACG AC-3', which corresponds to the IE gene sequence base numbers 1867 to 1886. These primers amplify a 202-bp segment of IE RNA.

**RT-PCR amplification of LAT and IE RNA**—The final reaction mixture contained 5  $\mu$ L of 10 $\times$  1-step buffer (400mM Tricine, 200mM KCl, 30mM MgCl<sub>2</sub>, and 37.5  $\mu$ g of bovine serum albumin/mL), 0.2mM of each dNTP, 20 units of recombinant RNase inhibitor, 25  $\mu$ L of thermostabilizing reagent, 10  $\mu$ L of GC amplification reagent,<sup>8</sup> 1 $\times$  RT *Taq* polymerase enzyme mix,<sup>1</sup> 0.9 $\mu$ M of the first primer (ie, LAT primer 1 or IE primer 1), 1  $\mu$ g of sample RNA, and sufficient RNase-free H<sub>2</sub>O to achieve a final reaction volume of 50  $\mu$ L. The 50- $\mu$ L reaction volume was overlaid with 20  $\mu$ L of light mineral oil. The cDNA synthesis was performed in an automated thermocycler<sup>d</sup> with an initial step of 60 minutes at 50°C followed by 94°C for 5 minutes. Then, 0.9 $\mu$ M of the second primer (ie, LAT primer 2 or IE primer 2) was added to each sample, and the amplification was continued with 50 cycles of 30 seconds at 94°C, 30 seconds at 65°C, and 1 minute at 68°C. A final step of 2 minutes at 68°C was included. The positive-control sample consisted of 1  $\mu$ g of FHV-1 RNA. Negative-control and digest-control samples were also included with each RT-PCR assay. Amplification products were electrophoresed on a 1.5% agarose gel containing ethidium bromide, photographed under ultraviolet illumination, and transferred<sup>12</sup> to nylon membranes.<sup>h</sup> The identity of PCR products was confirmed by hybridization to digoxigenin-labeled probes. One 20-mer oligonucleotide probe (LAT probe, 5'-TAA ACA CAA TTG CCA GCA GC-3') bound to the LAT sequence internal to the PCR primers. The other 20-mer oligonucleotide probe (IE probe, 5'-CAC ATC CGG

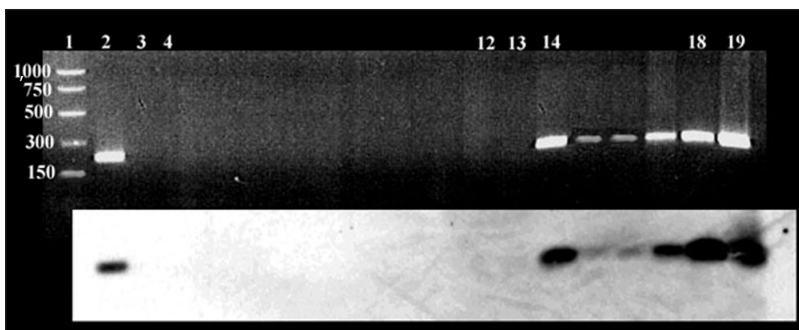


Figure 2—Results for agarose gel electrophoresis (top) and Southern blotting (bottom) of PCR amplification products of FHV-1 DNA obtained from corneal and trigeminal ganglia specimens of cats. Lanes were as follows: 1, DNA markers; 2, positive-control sample with a band at 224 bp; 3, digest-control sample; 4 to 12, specimens with from corneas of cats; 13, negative-control sample; and 14 to 19, specimens from trigeminal ganglia of cats. Notice the negative results for the corneal samples but the positive results for the trigeminal ganglia (ie, bands at 224 bp). Numbers on the left side are the number of base pairs.

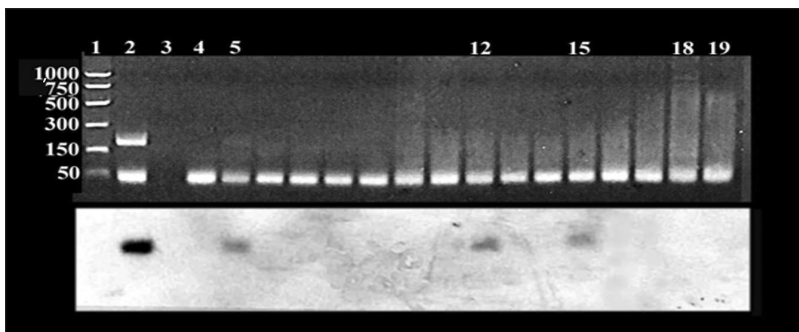


Figure 3—Results for agarose gel electrophoresis (top) and Southern blotting (bottom) of RT-PCR amplification products from the LAT primers of trigeminal ganglia specimens obtained from cats. Lanes were as follows: 1, DNA markers; 2, positive-control sample with a band at 200 bp; 3, digest-control sample; 4 to 18, trigeminal ganglia specimens obtained from cats; and 19, negative-control sample. Notice the negative results for lanes 4, 6 to 11, 13, 14, and 16 to 18, whereas lanes 5, 12, and 15 yielded positive results. The bands at 50 bp are primer dimers (an artifact) and do not represent a positive result. Numbers on the left side are the number of base pairs.

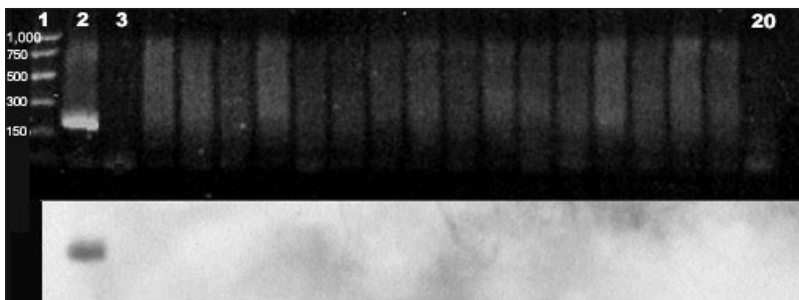


Figure 4—Results of agarose gel electrophoresis (top) and Southern blotting (bottom) of RT-PCR amplification products from the IE primers of trigeminal ganglia specimens obtained from cats. Lanes were as follows: 1, DNA markers; 2, positive-control sample with a band at 202 bp; 3, digest-control sample; 4 to 19, specimens of trigeminal ganglia obtained from cats; and 20, negative-control sample. Notice that all specimens of trigeminal ganglia had negative results. Numbers on the left side are the number of base pairs.



AGG GAC TAA AA-3') bound to the IE sequence internal to the IE primers. Limit of detection of RNA for the positive-control samples by use of the LAT primers was 3.125 ng, whereas by use of the IE primers it was 1.562 ng. Automated DNA sequencing<sup>1</sup> of the amplification products verified their specificity. Sequenced fragments each had 99% homology with the expected product.

## Results

Twenty-nine of 50 (58%) corneas obtained from group-1 cats and 16 of 42 (38%) corneas obtained from group-2 cats had positive results when tested for FHV-1 DNA, as evidenced by a band of the appropriate size that was confirmed by hybridization. Thus, 45 of 92 (49%) corneas had positive results for FHV-1 DNA. Sixteen of 50 (32%) trigeminal ganglia obtained from group-1 cats and 22 of 42 (52%) trigeminal ganglia obtained from group-2 cats had positive results when tested for FHV-1 DNA (Fig 2). Thus, 38 of 92 (41%) trigeminal ganglia had positive results for FHV-1 DNA.

Of 29 cats that had positive results for a corneal sample, 13 had positive results for only 1 cornea and 16 had positive results for both corneas. Of the 26 cats that had positive results for a trigeminal ganglia sample, 12 had positive results for only 1 trigeminal ganglion and 14 had positive results for both trigeminal ganglia.

Thirty-eight cats had tissues that yielded positive results when tested for FHV-1 DNA. Twelve cats had detectable FHV-1 DNA in their corneas only, whereas 9 cats had a positive result for the trigeminal ganglia and did not have detectable virus in their corneas. Seventeen cats had detectable FHV-1 DNA in their trigeminal ganglia and corneas.

Only samples with detectable FHV-1 DNA were subjected to the RT-PCR assays. None of the 29 group-1 corneas and none of the 10 group-2 corneas had detectable LATs. The trigeminal ganglia samples from group-2 cats did not have RNA of sufficient quality or quantity to perform the RT-PCR assay by use of the LAT primers, as indicated by the fact that feline RNA control glyceraldehyde 3-phosphate dehydrogenase could not be amplified. Four of 16 group-1 trigeminal ganglia had detectable LATs on the Southern blot of the RT-PCR products (Fig 3).

None of the 29 group-1 corneas and none of the 10 group-2 corneas had detectable IE transcripts. Trigeminal ganglia samples from group-2 cats did not have RNA of sufficient quality or quantity to perform the RT-PCR assay by use of the IE primers, as indicated by the fact that feline RNA control glyceraldehyde 3-phosphate dehydrogenase could not be amplified. None of the 16 trigeminal ganglia samples from group-1 cats had detectable IE transcripts (Fig 4).

## Discussion

To our knowledge, the study reported here documents for the first time that an RT-PCR assay that involved the use of LAT primers was able to detect LATs in cats that did not have clinical signs of ocular disease. The LATs were detected in 4 of 16 trigeminal ganglia that contained FHV-1 DNA. There were no positive results by use of the RT-PCR assay when the IE primers were used, indicating that the positive results

with the LAT primers were not false-positive results caused by amplification of the IE gene RNA. However, this did not definitively determine that those samples containing LATs represented purely latent infection. In another study,<sup>17</sup> LATs from herpes simplex virus-1 (HSV-1) were detected in 11 corneas obtained from humans. However, 2 of those corneas also had evidence of glycoprotein C transcription that indicated viral replication. Therefore, to definitively document latency, the samples in our study would need to be screened for evidence of other transcriptional activities, including glycoprotein C transcripts. Because of the limited amount of RNA available, those assays could not be performed.

We did not detect LATs in the corneas in the study reported here despite that fact that 45 of 92 (49%) had detectable amounts of FHV-1 DNA. The concentration of LATs in neurons is believed to be extremely high and has been estimated to be between  $10^4$  and  $10^5$  copies/cell.<sup>9</sup> However, it was suggested in 1 study<sup>18</sup> that the LAT promoter may be neuron-specific and that the amounts of LATs in nonganglionic cells, such as latently infected corneas, would be much lower.<sup>9</sup> Also, although some believe that the numbers of viral genomes and LAT RNA molecules appear to remain constant once latency is established,<sup>19</sup> mice experimentally infected with HSV-1 had a decreasing number of LAT-producing neurons as the postinoculation interval increased.<sup>20,21</sup> The assay used in the study reported here may have been unable to detect these lower amounts of LATs. As mentioned previously, results of 1 study<sup>17</sup> provided evidence of HSV-1 LATs within human corneal samples. In 9 of 11 corneal samples in that study, there was no evidence of glycoprotein C transcription, which suggested that HSV-1 may be maintained in a latent state within the cornea. In the study we conducted, we were not able to provide evidence that FHV-1 establishes latent infections within the cornea of cats. However, considerable evidence exists to support persistence of the herpesviral genome within the cornea. Feline herpesviral DNA has been detected by use of PCR assays in corneas from cats with corneal sequestra and eosinophilic keratitis as well as in cats without clinical signs of ocular disease.<sup>3,4</sup> In an outbreak of naturally developing FHV-1 infection in specific-pathogen-free cats,<sup>22</sup> FHV-1 DNA was consistently detected in the corneas, optic nerves, trigeminal ganglion, olfactory bulbs, and nasal turbinates of cats by use of a PCR assay, whereas FHV-1 could not be isolated in cell culture, suggesting a latent state. It has been documented by use of a PCR assay that HSV-1 DNA persists in human corneas with quiescent HSV infection.<sup>23</sup> Mice experimentally infected with HSV-1 can have detectable amounts of herpesviral DNA in corneal epithelial cells for up to 60 days after infection, yet infectious virus could only be isolated for up to 11 days after infection.<sup>24</sup> The persistence of HSV-1 in ocular tissues has also been documented through prolonged cocultivation on Vero monolayers of corneas harvested from experimentally infected mice that no longer had clinical signs of active ocular infection.<sup>25</sup> Finally, experimentally infected rabbits can have HSV-1 that is detectable only after prolonged culture of collagenase-digested corneas.<sup>26</sup> The inability to detect cell-free infec-

tious virus suggests that the virus remained in a latent state within those rabbit corneas. Both latent and actively replicating FHV-1 could be in the cornea simultaneously, although further studies will be needed to evaluate this hypothesis.

We did not identify IE transcripts in the trigeminal ganglia or corneal RNA samples by use of the RT-PCR assay performed with IE primers. A likely explanation is that FHV-1 IE RNA is found during limited stages of viral activation. In another study,<sup>27</sup> FHV-1 IE mRNA could not be detected during the early and late stages of viral activation. Two promoters alternatively regulate expression of the FHV-1 IE gene, and a negative regulatory element exists for both sites. It has been hypothesized<sup>28</sup> that the IE promoter and synthesis of IE mRNA are turned off after the immediate early phase. In contrast, equine herpesvirus-1 IE mRNA can be abundantly detected even during the early and late stages of viral transcription.<sup>28</sup> In another study,<sup>29</sup> investigators documented that the pattern of viral gene expression during reactivation is not the same as that seen during lytic infection of cell cultures. Instead of following the classic immediate-early, early, and late gene expression, early viral transcripts were detected before immediate-early transcripts during reactivation. This finding also suggests a limited period during which IE RNA may be detected.

Although we could not detect LATs within the corneal samples, the positive DNA results indicated viral involvement and may help explain the high incidence of cats with corneal disease associated with herpesvirus. The high percentage (49%) of corneal samples that had positive results for FHV-1 DNA confirms the results of another study<sup>4</sup> in which 46% of cats without clinical signs of ocular disease also had detectable amounts of FHV-1 DNA within their corneas. The 13 of 46 (28%) cats with positive results for only 1 corneal sample and 14 of 46 (30%) cats with positive results for only 1 trigeminal ganglion sample were interesting because recurrent ocular disease is typically unilateral for FHV-1<sup>30</sup> and HSV-1<sup>31</sup> infections. Also interesting were the 9 of 46 (20%) cats that had positive results for corneal samples without detectable FHV-1 DNA in the ipsilateral trigeminal ganglion. These samples may have indicated an early viral infection that had not yet caused clinical signs, a latent virus within the cornea, or a low-level viral infection that was not causing clinical signs. Some support does exist for the idea that HSV-1 can persist in a nonlatent form for an extended period.<sup>24,32</sup> A mutant form of HSV-1 can cause prolonged and productive infection in the nervous system of mice yet result in few clinical signs of disease.<sup>32</sup>

Assays involving RNA can be problematic because of the extremely labile nature of RNA. The quantity of RNA available in the study reported here was limited. In the future, use of a modified tissue-collection technique with immediate collection of the entire cornea after an animal is euthanized and storage of the cornea in liquid nitrogen would allow extraction of DNA and RNA. This should provide an increase in the quality and quantity of extracted RNA, which would allow evaluation for evidence of other FHV-1 transcriptional activities, including that of glycoprotein C

and thymidine kinase. Perhaps the use of microarray techniques would allow deduction of the pattern of transcriptional activity within these corneas. Gene expression in corneal tissues of cats could be explored in tandem by use of microarrays to examine cytokine production and other host cellular responses to FHV-1. These assays could also be applied to clinical samples, such as keratectomy specimens from patients with corneal sequestra or cytologic samples from patients with eosinophilic keratitis, to better define whether FHV-1 plays an active role in these conditions or is merely a bystander detected by highly sensitive PCR assays. The eventual determination of the transcriptional state of FHV-1 in the cornea of cats and its interaction with the host tissues will allow for improved therapeutic intervention and prevention or amelioration of FHV-1-associated corneal disease.

<sup>a</sup>RNAlater, Ambion, Austin, Tex.

<sup>b</sup>Tri-Reagent, Molecular Research Center Inc, Cincinnati, Ohio.

<sup>c</sup>Taq DNA polymerase, Promega Corp, Madison, Wis.

<sup>d</sup>PTC-200, Peltier thermal cycler, MJ Research, Watertown, Mass.

<sup>e</sup>Titanium One-Step RT-PCR kit user manual, Clontech, Palo Alto, Calif.

<sup>f</sup>RT-Titanium TaqEnzyme Mix, Clontech, Palo Alto, Calif.

<sup>g</sup>GC-Melt, Clontech, Palo Alto, Calif.

<sup>h</sup>S&S Nytran N, Schleicher & Schuell, Keene, NH.

<sup>i</sup>ABI 3700 dye terminator chemistry, Purdue Genomics Core Facility, West Lafayette, Ind.

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