Corneal ulceration associated with naturally occurring canine herpesvirus-1 infection in two adult dogs

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Case Description—An 8-year-old Labrador Retriever with diabetes mellitus in which bilateral phacoemulsification had been performed 3 weeks earlier was evaluated for acute onset of blepharospasm, and a 7-year-old Miniature Schnauzer with chronic immune-mediated thrombocytopenia was reevaluated for keratoconjunctivitis sicca that had been diagnosed 4 weeks earlier.

Clinical Findings—Dendritic corneal ulcerations were detected in both dogs. Canine herpesvirus-1 (CHV-1) was isolated from corneal swab specimens obtained during the initial evaluation of each dog and during recheck examinations performed until the ulcerations were healed. Canine herpesvirus-1 serum neutralization titers were detected in both dogs. Results of virus isolation from oropharyngeal and genital swab specimens were negative for both dogs. The isolated viruses were identified as CHV-1 via immunofluorescence, transmission electron microscopy, PCR assay, and gene sequencing. Negative controls for PCR assay and virus isolation included conjunctival swab specimens from 50 dogs without extraocular disease and corneal swab specimens from 50 dogs with corneal ulcers, respectively.

Treatment and Outcome—Lesions resolved in both dogs after topical administration of idoxuridine or trifluridine and discontinuation of topically administered immunosuppressive medications.

Clinical Relevance—To the authors’ knowledge, this is the first report of corneal ulcerations associated with naturally occurring CHV-1 infection and may represent local ocular recrudescence of latent CHV-1 infection. The viruses isolated were identified as CHV-1, and the morphology, antigenicity, and genotype were similar to those for CHV-1 isolates obtained from a puppy that died from systemic CHV-1 infection. (J Am Vet Med Assoc 2006;229:376–384)

An 8-year-old castrated male Labrador Retriever was evaluated for acute blepharospasm and epiphora of the left eye. Diabetes mellitus had been diagnosed in the dog 8 months earlier, and the dog was receiving neutral protamine Hagedorn (NPH) insulin (15 units, SC, q 12 h). Bilateral cataract phacoemulsification and intraocular lens implantation had been performed 3 weeks prior to evaluation, and neomycin-polymyxin B-dexamethasone ointment was being topically administered in both eyes every 6 hours. One week after surgery, the dog developed glaucoma bilaterally and was being treated with latanoprost solution (right eye, q 12 h; left eye, q 24 h) and timolol-dorzolamide solution (both eyes, q 8 h). No abnormalities were detected on CBC, serum biochemical analyses, and urinalysis performed at the time of surgery. Intermittent blepharospasm was observed in the left eye during ophthalmic examination. Two superficial linear corneal ulcers that retained fluorescein stain were detected in the left eye. The ulcerations had discrete margins, a dendritic pattern, and were located in the temporal, para-axial aspect of the cornea (Figure 1). The ulcerations were not associated with corneal edema or leukocyte infiltration. Conjunctival hyperemia, chemosis, and aqueous flare were not observed in either eye, and the remainder of the ophthalmic examination, including Schirmer I tear test results and values for intraocular pressures measured by applanation tonometry, was considered normal. No abnormalities were detected on physical examination.

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ABBREVIATIONS

CHV-1 Canine herpesvirus-1
FHV-1 Feline herpesvirus-1
HSV Herpes simplex virus

Figure 1—Photograph of the left eye of an 8-year-old dog evaluated for acute blepharospasm. Two superficial dendritic corneal ulcers that retained fluorescein stain were detected.
Corneal swab specimens were obtained for aerobic bacterial culture and virus isolation from the areas of ulceration prior to topical administration of anesthetic. Corneal scrapings were performed after topical application of anesthetic for cytologic evaluation. No growth was detected on aerobic bacterial culture; CHV-1 was isolated from corneal swab specimens. Cytologic examination of corneal scrapings revealed nonkeratinized squamous epithelial cells; no inflammatory cells or viral inclusions were observed. Administration of neomycin-polymyxin B-dexamethasone ointment was discontinued in the left eye. In addition to the previously prescribed medications for treatment of glaucoma, topical administration of 0.1% idoxuridine solution (6 to 8 times daily for 48 hours and q 6 h thereafter), neomycin-polymyxin B-bacitracin ointment (q 6 h), and 0.03% flurbiprofen solution (q 8 h) in the left eye was initiated, and the dog was discharged.

Two weeks after the initial evaluation, the dog was reevaluated. The owner reported occasional, but reduced, blepharospasm in the left eye. The 2 linear corneal ulcers could still be detected and retained fluorescein stain, but the length and width of both ulcers appeared to have decreased, and they were no longer associated with branches. The remainder of the ophthalmic examination was unchanged from the previous evaluation. Canine herpesvirus-1 was again isolated from a swab specimen obtained from the corneal ulcerations. Blood was collected, and serum was obtained for a serum neutralization test for CHV-1, results of which were positive (1:1,024). Topical administration of cyclosporine ointment (both eyes, q 12 h) and neomycin-polymyxin B-dexamethasone ointment (both eyes, q 8 h). Immune-mediated thrombocytopenia had been diagnosed in the dog 2 months earlier, and the dog was receiving prednisone (1.0 mg/kg [0.45 mg/lb], PO, q 12 h) and famotidine (0.5 mg/kg [0.23 mg/lb], PO, q 12 h). Results of a CBC and urinalysis were considered normal. Abnormalities detected on serum biochemical analyses included high activities of serum alkaline phosphatase (554 U/L; reference range, 12 to 122 U/L), γ-glutamyltransferase (137 U/L; reference range, 0 to 10 U/L), alanine transaminase (992 U/L; reference range, 25 to 106 U/L), and aspartate transaminase (64 U/L; reference range, 16 to 50 U/L). Intermittent blepharospasm was observed in the right eye during ophthalmic examination. Two superficial corneal ulcers that retained fluorescein stain were detected in the right eye (Figure 2). Both ulcers were linear and had a dendritic pattern. The ulcers were not associated with corneal edema or leukocyte infiltration. One ulcer was located in the axial aspect of the cornea, and the other was located in the temporal para-axial aspect of the cornea. Results of Schirmer I tear tests were normal (right eye, 15 mm/min; left eye, 20 mm/min), and conjunctival hyperemia, chemosis, and aqueous flare were not detected. The remainder of the ophthalmic examination findings, including values for intraocular pressure measured by applanation tonometry, and physical examination findings were considered normal.

Corneal swab specimens for aerobic bacterial culture and virus isolation were obtained from the ulcerations prior to topical application of anesthetic. Swab specimens were also obtained from the vaginal and oropharyngeal mucosa for virus isolation. After topical application of anesthetic, corneal scrapings were obtained from the ulcers for cytologic evaluation.

A 7-year-old spayed female Miniature Schnauzer was reevaluated for bilateral keratoconjunctivitis sicca that had been diagnosed 4 weeks earlier. At that time, conjunctivitis and mucoid ocular discharge were detected bilaterally and results of Schirmer I tear tests were 9 and 8 mm/min in the right and left eyes, respectively (reference range, ≥ 15 mm/min = normal tear production). The dog was being treated with 0.2% cyclosporine ointment (both eyes, q 12 h) and neomycin-polymyxin B-dexamethasone ointment (both eyes, q 8 h). Immune-mediated thrombocytopenia had been diagnosed in the dog 2 months earlier, and the dog was receiving prednisone (1.0 mg/kg [0.45 mg/lb], PO, q 12 h) and famotidine (0.5 mg/kg [0.23 mg/lb], PO, q 12 h). Results of a CBC and urinalysis were considered normal. Abnormalities detected on serum biochemical analyses included high activities of serum alkaline phosphatase (554 U/L; reference range, 12 to 122 U/L), γ-glutamyltransferase (137 U/L; reference range, 0 to 10 U/L), alanine transaminase (992 U/L; reference range, 25 to 106 U/L), and aspartate transaminase (64 U/L; reference range, 16 to 50 U/L). Intermittent blepharospasm was observed in the right eye during ophthalmic examination. Two superficial corneal ulcers that retained fluorescein stain were detected in the right eye (Figure 2). Both ulcers were linear and had a dendritic pattern. The ulcers were not associated with corneal edema or leukocyte infiltration. One ulcer was located in the axial aspect of the cornea, and the other was located in the temporal para-axial aspect of the cornea. Results of Schirmer I tear tests were normal (right eye, 15 mm/min; left eye, 20 mm/min), and conjunctival hyperemia, chemosis, and aqueous flare were not detected. The remainder of the ophthalmic examination findings, including values for intraocular pressure measured by applanation tonometry, and physical examination findings were considered normal.

Corneal swab specimens for aerobic bacterial culture and virus isolation were obtained from the ulcerations prior to topical application of anesthetic. Swab specimens were also obtained from the vaginal and oropharyngeal mucosa for virus isolation. After topical application of anesthetic, corneal scrapings were obtained from the ulcers for cytologic evaluation.

Figure 2—Photograph of the right eye of a 7-year-old dog evaluated for bilateral keratoconjunctivitis sicca that had been diagnosed 4 weeks earlier. Two superficial dendritic corneal ulcers that retained fluorescein stain were detected.
Blood was collected, and serum was obtained for a serum neutralization test for CHV-1; results were positive (1:1,024). Canine herpesvirus-1 was isolated from the corneal swab specimen. Results of virus isolation from vaginal and oropharyngeal mucosa specimens were negative. *Staphylococcus warneri* was isolated from enrichment of the aerobic bacterial culture of the cornea and was considered most likely to be a contaminant. Cytologic evaluation of the corneal scraping revealed nonkeratinized and keratinized squamous epithelial cells; no inflammatory cells or viral inclusions were observed. Treatment with 0.1% idoxuridine solution (right eye, 6 to 8 times daily for 48 hours and q 6 h thereafter) and L-lysine (500 mg, PO, q 12 h) was initiated. Topical administration of neomycin-polymyxin B-dexamethasone ointment bilaterally was discontinued; topical administration of 0.2% cyclosporine ointment bilaterally was continued.

Ten days after the initial examination, intermittent blepharospasm was reported by the owner and was observed in both eyes during examination. Four dendritic corneal ulcers of various lengths were detected scattered over the corneal surface of the right eye (Figure 3). A single dendritic corneal ulcer was detected in the left eye adjacent to the ventrotemporal limbus (Figure 4). Canine herpesvirus-1 was isolated from separate corneal swab specimens obtained from areas of ulceration in both eyes and processed independently for virus isolation. Results of a serum neutralization test for CHV-1 were positive (1:512). Treatment with 0.1% idoxuridine solution was continued in the right eye and initiated in the left eye (6 to 8 times daily for 48 hours and q 6 h thereafter). Topical administration of 0.2% cyclosporine ointment and oral administration of L-lysine were also continued. Three weeks after the initial examination, the corneal ulcerations in both eyes had not changed. Canine herpesvirus-1 was again isolated from separate swab specimens obtained from corneal ulcerations in both eyes, and results of a serum neutralization test for CHV-1 were positive (1:1,024). Topical administration of 1% trifluridine solution (6 to 8 times daily for 48 hours and q 6 h thereafter) was ini-
tiated in both eyes, and oral administration of L-lysine was continued. Treatment of both eyes with cyclosporine ointment and idoxuridine solution was discontinued. Four weeks after the initial examination, the length and the number of branches of the ulcerations in both eyes appeared to have decreased, compared with the previous examination. Canine herpesvirus-1 was again isolated from corneal swab specimens obtained from both eyes, and results of a serum neutralization test for CHV-1 were positive (1:1,024). Treatment was continued. Five weeks after the initial examination, the ulceration in the left eye retained fluorescein stain, but the length and width of the ulcer appeared to have decreased further. Linear dendritic corneal epithelial opacities corresponding to areas of previous ulceration were detected in the right eye. These corneal opacities retained rose bengal stain but not fluorescein stain. Canine herpesvirus-1 was isolated from separate swab specimens obtained from the ulceration in the left eye and the epithelial opacities in the right eye. Results of a serum neutralization test for CHV-1 were positive (1:312). Treatment was continued unaltered.

Seven weeks after the initial examination, the ulceration in the left eye and dendritic epithelial opacities in the right eye had resolved and neither cornea retained fluorescein or rose bengal stain. Marked, diffuse epithelial pigmentation was detected in both eyes (Figure 3). Results of Schirmer I tear tests in both eyes were within reference range (right eye, 18 mm/min; left eye, 20 mm/min). Results of virus isolation on corneal and conjunctival swab specimens were negative for both eyes; results of a serum neutralization test for CHV-1 were positive (1:1,536). Topical administration of trifluridine solution was continued for 2 additional weeks and then discontinued. Topical administration of 0.2% cyclosporine ointment was resumed (both eyes, q 12 h), and oral administration of L-lysine was continued. Ten weeks after the initial examination, the ulcerations and dendritic epithelial opacities in both eyes remained resolved. The epithelial pigmentation had improved mildly, and Schirmer I tear test values in both eyes had decreased (10 mm/min). Through the clearing corneal pigment, punctate accumulations of refractile white opacities were visible in the anterior stroma of the axial aspect of the cornea in both eyes, compatible with lipid or mineral deposition. Conjunctival swab specimens were obtained from both eyes for virus isolation; results were negative. Results of a serum neutralization test for CHV-1 were positive (1:1,024). Topical administration of 0.2% cyclosporine ointment and oral administration of L-lysine were continued, and topical administration of artificial tear gel (both eyes, q 4 h) was initiated.

Recheck examinations were performed approximately every 4 weeks during the subsequent 14 months. No recurrence of clinical signs, corneal ulcers, or other ophthalmic abnormalities was observed during this period. Fourteen weeks after the initial evaluation, Schirmer I tear test values in both eyes were within reference range, and values in both eyes remained > 15 mm/min at each subsequent examination. The corneal pigmentation gradually resolved over 16 weeks; however, the refractile, white stromal deposits persisted (Figure 3). Oral administration of L-lysine and topical administration of 0.2% cyclosporine ointment were continued throughout this period. Serum neutralization tests for CHV-1 were performed during each recheck, and results varied but remained positive (range, 1:192 to 1:1,024). Treatment with lamotrigine and prednisone for immune-mediated thrombocytopenia was continued; the dose of prednisone was gradually tapered.

In the dogs reported here, sterile polyester-tipped swabs were used for all virologic sample collections. In all instances, corneal swab specimens were collected after fluorescein and rose bengal staining but prior to topical application of anesthetic. Thorough irrigation of the ocular surface was performed prior to collection of swab specimens to remove as much stain as possible. Swab specimens were immediately placed in viral transport medium (ie, Leibovitz L-15 medium with 7.5% bovine serum albumin solution, 1% gentamicin sulfate solution, and 1% amphotericin B solution). Samples were delivered to the laboratory for virologic processing within 1 hour of collection. Virus isolations were performed with Madin-Darby canine kidney cells in minimum essential medium-E with 10% fetal bovine serum, 5% serum replacement solution, 2% penicillin-streptomycin solution, 1% amphotericin B sulfate solution, and 1% gentamicin sulfate solution. Cultures were incubated at 37°C, checked at 24-hour intervals for growth, subcultured every 3 to 7 days, and held for 21 days.

Viruses isolated from corneal swab specimens of both dogs during the initial evaluation and during recheck examinations until the ulcerations were healed had growth characteristics and cytopathic effects typical of herpesviruses. Staining of cell cultures with anti–CHV-1 polyclonal antisera conjugated to fluorescein isothiocyanate yielded positive results, and the viruses had morphologic characteristics compatible with CHV-1 when viewed with transmission electron microscopy. To more conclusively identify
Figure 5—Transmission electron photomicrograph of the CHV-1 isolate obtained from the dog in Figure 1. Bar = 100 nm.

the viruses, viral DNA was extracted from 200 µL of the cell culture supernatant by use of a kit. The cell culture supernatant from the initial corneal virus isolation from each dog was used for each virus. Three separate PCR assays, consisting of 2 CHV-1–specific and 1 panherpesviral primer pairs, were performed to amplify a 350–base pair product from the immediate-early gene, a 110–base pair product from the thymidine kinase gene, and a 700–base pair product from the DNA polymerase gene, respectively. Positive controls consisted of DNA extracted from CHV-1 grown in culture and isolated from a puppy that died because of systemic CHV-1 infection. Negative controls included water blanks extracted in parallel with the dogs’ samples and a negative (sterile water) PCR assay control. Reaction conditions for all primer pairs consisted of an initial denaturing temperature of 95°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute, and extension at 72°C for 1 minute. Products were analyzed by gel electrophoresis on a 1.5% agarose gel followed by ethidium bromide staining.

Both clinical viral isolates and the positive control isolate tested positive by PCR assay by use of the panherpesviral- and CHV-1–specific primer pairs. To determine whether the viruses were CHV-1 or closely related viruses that were amplified by use of the CHV-1 specific primer pairs, all resultant PCR assay products, including positive controls, were cloned and sequenced bidirectionally. The PCR assay products were cloned by use of a cloning kit and sequenced bidirectionally by use of M13 forward and reverse primers. All products were compared with published sequences by use of the Basic Local Alignment Search Tool (BLAST). Resultant sequences had 100% sequence identity with CHV-1. These results strongly support the identification of the viruses as CHV-1, despite the potential for there to be some sequence difference outside of the products sequenced.

To determine whether CHV-1 was likely the primary pathogen in both dogs, an ocular virologic survey was performed. This survey consisted of virus isolation performed on corneal swab specimens from 50 adult dogs (ie, > 1 year old) evaluated for various corneal ulcers and PCR assays with CHV-1–specific primer pairs performed on conjunctival swab specimens collected from 50 randomly chosen adult dogs (ie, > 1 year old) without clinical evidence of extraocular disease. Sterile technique was used to obtain swab specimens. Lesions in dogs with corneal ulcers did not resemble the lesions detected in the 2 dogs reported here (ie, dendritic corneal ulcers) but were chosen because ulcers in these dogs represented a spectrum of corneal ulcer types and etiologies commonly observed in dogs, including septic (n = 8) and sterile (4) stromal corneal ulcers, spontaneous chronic corneal epithelial defects or indolent ulcers (10), postanesthetic corneal ulcers (10), acute idiopathic linear and geographic ulcers presumed to have developed secondary to trauma (10), corneal ulcers associated with entropion or ectopic cilia (4), and corneal ulcers associated with corneal degeneration or endothelial decompensation (4). Dogs without clinical evidence of extraocular disease were evaluated for various non–ocular-related problems. Results of slitlamp biomicroscopic examinations in those dogs were considered normal, and the dogs had not received topically administered ophthalmic medications. Informed consent was obtained from owners of all 50 randomly chosen dogs prior to sample collection. Conjunctival swab specimens were placed in sterile anticoagulant-free tubes with 300 µL of sterile saline (0.9% NaCl) solution. After vortexing thoroughly, 200 µL of this saline solution was used for DNA extraction and tested via PCR assay by use of the previously described DNA extraction methods and CHV-1–specific primer pairs. A 214–base pair fragment of the canine histone 3.3 gene was amplified to assess the adequacy and integrity of DNA extracted from all swab specimens. None of the conjunctival samples from dogs without clinical extraocular disease tested positive via PCR assay for either CHV-1–specific PCR reaction, and a 214–base pair histone DNA fragment was amplified from all samples. Results of all virus isolations of corneal swab specimens from dogs with corneal ulcers were negative. These results suggested that CHV-1 was the primary pathogen in the 2 dogs reported here and not a virus that could be found associated with extraocular tissues of dogs without clinical evidence of extraocular disease or as a secondary infection or reactivation associated with corneal ulceration. Virus isolation is not the most sensitive means of detecting a virus; however, it was chosen to determine whether actively replicating CHV-1 could be recovered from other dogs with corneal ulcers, as was performed in the 2 dogs of this report.

Discussion

Canine herpesvirus-1 is a member of subfamily Alphaherpesvirinae and genus Varicellovirus. Canine herpesvirus-1 is antigenically related to FHV-1 and HSV and possesses biological and pathogenic properties similar to alphaherpesviruses affecting other species. The host range of CHV-1 includes domestic and wild canids.
monly associated with systemic, often fatal, fetal and neonatal infections in domestic dogs.\textsuperscript{15,18} These infections are characterized by disseminated necrosis and hemorrhage of parenchymal organs.\textsuperscript{15,17} Ocular lesions in immature dogs include nonulcerative keratitis, panuveitis, optic neuritis, retinitis, and retinal dysplasia.\textsuperscript{16,19} Infections in mature dogs are believed to be primarily subclinical; however, CHV-1 has been detected in adult dogs with upper respiratory tract disease, genital mucositis, and conjunctivitis.\textsuperscript{20,28} The causative or contributory role of CHV-1 in these disease processes is not clear.

A defining characteristic of alphaherpesviruses is the ability to induce lifelong latent infections.\textsuperscript{29} Several alphaherpesviruses related to CHV-1, including human HSV, FHV-1, and bovine herpesvirus-1, are associated with well-characterized latent infection and recurrent ocular disease syndromes.\textsuperscript{30,31} These viruses may establish latent infections in the sensory ganglia and possibly within ocular tissues, with the trigeminal ganglia believed to be the primary source of recurrent, clinical ocular disease.\textsuperscript{32,33} Reactivation of latent infections may occur spontaneously or in association with various stimuli, including local or systemic immunosuppression, with resultant neuronal spread of the virus to the cornea.\textsuperscript{33,36,40,41} Superficial, dendritic corneal ulcerations are common with recrudescent HSV\textsuperscript{31,42} and FHV-1\textsuperscript{33} ocular infection and are similar to lesions observed in the dogs of this report.

Evidence for latent CHV-1 infections in adult dogs has been detected in various tissues by use of PCR assay.\textsuperscript{19,35} The lumbarosacral ganglia, tonsils, parotid salivary glands, and liver were reported as the most common sites of CHV-1 latency in 1 study\textsuperscript{3} of naturally infected dogs; however, viral DNA was also identified in the trigeminal ganglia. Canine herpesvirus-1 DNA was identified in the trigeminal ganglia and retropharyngeal lymph nodes of 100% and 87.5% of dogs, respectively, following recovery from acute experimental infection.\textsuperscript{3} Canine herpesvirus-1 DNA, suggestive of viral latency, has also been detected in the trigeminal ganglia of red foxes following experimental infection.\textsuperscript{44}

Latent CHV-1 reaction has been detected in puppies and adult dogs following natural and experimental CHV-1 infection.\textsuperscript{40,46} Experimental immunosuppression with prednisolone administered systemically resulted in viral shedding without clinical signs.\textsuperscript{46} Canine herpesvirus-1 was isolated from nasal, oral, genital, and ocular secretions as early as 5 days after initiating corticosteroid treatment, and virus was isolated from specimens obtained from the nasal mucosa and tonsils after death.\textsuperscript{48,49} Focal mononuclear cell infiltration and nerve cell degeneration were detected in the trigeminal and lumbarosacral ganglia of some dogs, suggesting that these were sites of reactivating CHV-1 latent infection.\textsuperscript{45} All latently infected dogs had low serum neutralization titers prior to treatment, and titers in some dogs increased following immunosuppression.\textsuperscript{45}

Circumstantial descriptions of latent CHV-1 infection reactivation have also been reported.\textsuperscript{32,33,45} Canine herpesvirus-1 DNA was detected in a conjunctival biopsy specimen from a dog with chronic superficial keratitis 1 week after strontium 90 radiotherapy, and it was postulated that this finding represented latent infection reactivation associated with the treatment.\textsuperscript{46} In adult dogs, death has been attributed to CHV-1 infections in association with parvovirus infections and may represent latent herpetic infection reactivation secondary to parvoviral immunosuppression.\textsuperscript{22} Canine herpesvirus-1 has also been isolated from recurrent vesicular lesions of the canine genital tract, findings suggestive of viral latency and reactivation.\textsuperscript{37}

Results of virus isolation tests performed on specimens obtained from the genital and oropharyngeal mucosa, sites from which CHV-1 was recovered most frequently following experimental viral reactivation,\textsuperscript{42,46} were negative in the dogs of this report. In 1 dog reported here, results of virus isolation tests performed on specimens obtained from the conjunctiva of the contralateral (unaffected) eye were also negative. These negative results indicate that virus was either not present, present in small quantities, present in a nonreplicating form, or present at the time of sampling but that viral viability was lost between the time of collection and the time of virus isolation testing.\textsuperscript{34}

Virus isolation tests performed at different chronologic points or the use of more sensitive methods for viral detection, such as PCR assay, may have yielded different results.\textsuperscript{34,46} Canine herpesvirus-1 was repeatedly isolated from swab specimens obtained from the affected corneas until the lesions had resolved, and results of virus isolation tests performed on corneal and conjunctival swab specimens were repeatedly negative following healing of the corneal ulcers in both dogs. The observed corneal lesions may have resulted from acute CHV-1 infection; however, the virologic results, clinical history, and extrapolations from knowledge of the behavior of other alphaherpesviruses are more suggestive of local ocular recrudescence of a latent infection.

In the dogs reported here, reactivation of a latent CHV-1 infection with subsequent corneal infection and ulceration may have resulted from various etiologies. The mechanisms by which latent herpetic infections are reactivated are not completely understood; however, many of the stimuli that induce viral reactivation promote inflammation or suppress the host immune response.\textsuperscript{46} Ocular surgery, administration of systemic and ophthalmic immunosuppressive medications (eg, corticosteroids and cyclosporine), and administration of ophthalmic medications for treatment of glaucoma (eg, latanoprost) have been associated with reactivation of latent herpetic infections in various species.\textsuperscript{46,49,50} Systemic administration of immunosuppressive medications such as corticosteroids has repeatedly been found to induce reactivation of latent CHV-1 infection in dogs.\textsuperscript{42,46} In addition to their immunomodulating activities, corticosteroids may also promote reactivation of latent herpesviruses by activating viral and host cell gene expression.\textsuperscript{51} Immune-mediated thrombocytopenia, diabetes mellitus, and stresses associated with hospitalization induce altered immune system function\textsuperscript{52,53} and may have contributed to an impaired ability to suppress the viral infection in the dogs reported here. Both dogs had several potential causes of altered immune system function, and 1 cause
or a combination of those causes may have contributed to viral reactivation.

Clinical resolution of corneal ulcers was detected in both dogs after topical administration of antiviral medications (ie, idoxuridine and trifluridine) and discontinuation of topically administered immunosuppressive medications (ie, dexamethasone and cyclosporine). The efficacy of those antiviral medications against ocular CHV-1 infections has not been established; however, these medications have broad-spectrum activity in vivo and in vitro against other alphaherpesviruses. One dog also received L-lysine, which has been found to reduce the in vitro replication of FHV-1 and HSV, decrease viral shedding in cats following experimentally induced reactivation of latent FHV-1 infections, reduce the severity of conjunctivitis in cats after primary FHV-1 infection, accelerate recovery from natural HSV infection in humans, and suppress natural HSV infection recurrence in humans.

In one of the dogs reported here, healing of the corneal ulcers was observed after topical administration of idoxuridine and discontinuation of topically administered dexamethasone. Flurbiprofen was also administered topically in this dog. This medication was used for control of postoperative uveitis after phacoemulsification, has been variably found to have a virus strain with specific corneal virulence that has been found to reduce the in vitro replication of FHV-1 and HSV, decrease viral shedding in cats following experimentally induced reactivation of latent FHV-1 infections, reduce the severity of conjunctivitis in cats after primary FHV-1 infection, accelerate recovery from natural HSV infection in humans, and suppress natural HSV infection recurrence in humans.

In one of the dogs reported here, healing of the corneal ulcers was observed after topical administration of idoxuridine and discontinuation of topically administered dexamethasone. Flurbiprofen was also administered topically in this dog. This medication was used for control of postoperative uveitis after phacoemulsification, has been variably found to have reduced potential to exacerbate herpetic corneal disease in comparison with corticosteroids administered topically, and inhibits HSV replication in vitro.

Corneal lesions in the other dog progressed for the first 3 weeks despite treatment with idoxuridine and L-lysine and discontinuation of topically administered dexamethasone. The corneal ulcerations resolved after topical administration of cyclosporine was discontinued and trifluridine was added to the treatment regimen. The refractory nature of the corneal lesions to idoxuridine treatment may have been associated with topical administration of cyclosporine, viral resistance to idoxuridine, or systemic administration of prednisone. Topical administration of cyclosporine has been found to prevent the resolution of herpetic keratitis in humans, despite appropriate antiviral treatment. In that report, corneal ulcerations healed after discontinuation of cyclosporine. Idoxuridine-resistant strains of CHV-1 have recently been identified and possess mutations of the viral thymidine kinase gene. Systemic administration of prednisone may also have contributed to the delayed resolution of the ulcerations and ideally would have been discontinued during treatment of the ocular infection; however, control of the dog’s immune-mediated thrombocytopenia necessitated continued administration throughout treatment of the ophthalmic lesions.

The high prevalence of herpesvirus infections among individuals of many species with nonclinical infections makes determination of an isolated herpesvirus’ role in a particular disease process problematic. This has been the situation in cats with FHV-1 ocular disease in which determination of incidental versus causative infection is difficult. On the basis of detection of virus in ocular tissues, FHV-1 has been historically speculated to be the cause of various corneal diseases in cats. In studies of cats without extracocular disease, however, FHV-1 DNA has been detected in 3.9% to 48.9% of corneas. These findings have confounded the conclusions of other reports attributing the cause of particular corneal lesions to FHV-1. Alphaherpesviruses may also be present in tissues as a reactivation of a latent infection associated with sensory nerve stimulation. This has been detected with corneal ulceration and resultant trigeminal nerve stimulation and must be considered prior to attributing a lesion to an isolated herpesvirus.

Results of serologic studies indicate that 40% to 93% of the canine population is seropositive for CHV-1. Considering the biological properties of related alphaherpesviruses, it is reasonable to assume that most of these dogs are latently infected. Serologic studies of dogs with ocular disease suggested that CHV-1 was the primary pathogen involved in the occurrence or persistence of corneal ulcerations in the 2 dogs and was not present as an incidental infection associated with extracocular tissues of dogs or a secondary reactivation resultant from corneal ulceration. However, only a limited number of dogs were sampled, and additional studies are required to confirm these results.

The pattern of age-dependent disease severity observed with CHV-1 infection is common within subfamily Alphaherpesvirinae. Severe systemic infections in neonates and relatively mild, recurrent mucosal lesions in adults are typical of these viruses, and the identification of ocular disease associated with latent CHV-1 reactivation in adult dogs could be considered an expected finding. To the authors’ knowledge, the 2 dogs reported here are the first documented cases of corneal ulceration caused by naturally occurring CHV-1 infection in adult dogs. Thorough examination of dogs with blepharospasm of unknown cause with adequate magnification, appropriate corneal stains (ie, fluorescein and rose bengal stains), and vigorous flushing of the corneal surface to remove staining artifacts is recommended for detection of dendritic corneal ulcers. As in other species, local and systemic immunosuppression may be important in the pathogenesis of herpetic corneal ulceration in dogs, and in the dogs reported here, resolution of clinical lesions was detected only after topical administration of immunosuppressive medications was discontinued. The viruses isolated were identified as CHV-1, and their morphology, antigenicity, and genotype were similar to those in CHV-1 isolates obtained from a puppy that died because of systemic CHV-1 infection; however, a virus strain with specific corneal virulence cannot be ruled out. The 2 dogs of this report were originally evaluated for corneal ulcers 6 weeks apart; however, other geographic or temporal associations between the dogs and contact between the dogs or their owners were not identified.
References


44. Reubel GH, Pekin J, Wright J, et al. Cytocorticoid treat-
63. Maggs DJ, Nasissse MP, Kass PH. Efficacy of oral supplemen-