Use of nested polymerase chain reaction to identify feline herpesvirus in ocular tissue from clinically normal cats and cats with corneal sequestra or conjunctivitis

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Objective—To use a nested polymerase chain reaction (PCR) to detect feline herpesvirus (FHV-1) DNA in conjunctiva or cornea from clinically normal cats and cats with conjunctivitis or corneal sequestra.

Samples—Conjunctival snip biopsy specimens from 50 cats with conjunctivitis and 50 clinically normal cats; 28 keratectomy specimens from 26 cats with sequestra, and 13 specimens from clinically normal cats.

Procedure—Tissue specimens were digested, and FHV-1 DNA was amplified, using a double round of PCR. Products were visualized by use of agarose gel electrophoresis.

Results—Polymerase chain reaction was positive in 27 of 50 (54%) conjunctival specimens from cats with conjunctivitis and 6 of 50 (12%) specimens from clinically normal cats. Difference in the results between cats with conjunctivitis and clinically normal cats was statistically significant. Polymerase chain reaction was positive in 5 of 28 (18%) corneal specimens from cats with sequestra and 6 of 13 (46%) clinically normal cats. Distribution of positive results between clinically normal cats and those with sequestra was not significant.

Conclusion—Cats with conjunctivitis were more likely to have a positive PCR result than were clinically normal cats, making it likely that FHV-1 was associated with the disease state. Herpesvirus DNA could not be detected in most corneas from cats with sequestra.

Clinical Relevance—Polymerase chain reaction is a useful clinical test for identifying FHV-1 DNA in cats with conjunctivitis, yielding greater sensitivity over that of currently available tests. Herpesvirus may be less of a cause of corneal sequestration in the commonly affected breeds, Himalayan and Persian, than other factors, such as lagophthalmos or corneal metabolic defects. (*Am J Vet Res* 1997;58:338–342)

Feline herpesvirus 1 (FHV-1) ocular infection is one of the most common ophthalmic diseases of cats. Clinical manifestations include conjunctivitis, corneal ulcers, stromal keratitis, corneal sequestration, and keratoconjunctivitis sicca.[1] Conjunctivitis is probably the most common feline clinical ophthalmic disorder facing clinicians, although determining the underlying cause can be extremely difficult. In a recent study of 91 cats with chronic conjunctivitis, only 19% had positive results of an FHV-1 fluorescent antibody test or virus culture, and 18% had positive results of a *Chlamydia psittaci* fluorescent antibody test.[2]

Corneal sequestration is a condition unique to cats, in which the corneal stroma undergoes necrosis, with resulting areas of black discoloration accompanied by variable degrees of stromal keratitis. A breed predilection for Himalayan and Persian, and to a lesser degree, Siamese, has been reported.[3-5] The condition has been associated with FHV-1 infection, experimentally induced[6] and naturally acquired,[1,5] although results of routine diagnostic tests, using virus isolation and fluorescent antibody detect FHV-1, are often negative.

Recently, the polymerase chain reaction (PCR) has been used to amplify specific portions of microbial DNA, allowing identification of minute amounts of target DNA in specimens.[7] The PCR has been used to identify FHV-1 in tissues and secretions and was found to be more sensitive than virus isolation.[8,9]

The objective of the study reported here was to evaluate conjunctival biopsy specimens from cats with acute or chronic conjunctivitis and from cats with clinically normal eyes, using a nested PCR to determine whether FHV-1 DNA was present. We also tested and compared corneal specimens from cats with corneal sequestration with those from clinically normal cats.

Materials and Methods

Conjunctiva—Conjunctival snip biopsy specimens of approximately 2 × 2 mm were obtained from 100 cats after application of a topical anesthetic. Cats were allotted to 2 groups of 50 cats each. Tissue specimens were placed in phosphate-buffered saline solution and frozen at −20 C until tested.

Group 1 comprised cats with conjunctivitis, either acute (< 2 weeks, n = 21) or chronic (> 2 weeks, n = 29). Breeds included domestic shorthair (32), Persian (7), Himalayan (4), domestic longhair (2), Burmese (1), Rex (1), Manx (1), Siamese (1) and Maine Coon (1). There were 25 neutered males, 21 spayed females, 3 sexually intact males, and 1 sexually intact female. Age ranged from 7 months to 15 years, with a mean of 4 years. Three cats were from research colonies, and 47 cats were client-owned pets. Specimens were collected from the affected eye; in instances in which specimens were collected from both eyes, only the eye judged to be worse clinically was included in the study.

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