Assessment of infectious organisms associated with chronic rhinosinusitis in cats

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Objective—To determine detection rates for feline herpesvirus type 1 (FHV-1), *Mycoplasma* spp, fungi, and bacteria in flush samples and biopsy specimens from the nasal cavities of cats with and without chronic rhinosinusitis (CRS).

Design—Prospective study.

Animals—10 CRS-affected cats and 7 cats without signs of respiratory tract disease.

Procedures—Nasal flush samples and biopsy specimens were collected from all cats for bacterial (aerobic and anaerobic), fungal, and mycoplasmal cultures; additional biopsy specimens were collected for virus isolation and polymerase chain reaction (PCR) assay (to detect FHV-1 DNA).

Results—Aerobic bacteria were detected in flush samples from 5 of 7 control cats; culture of flush samples from CRS-affected cats yielded aerobic bacteria (9/10 cats), anaerobic bacteria (3/10), and *Mycoplasma* spp (2/10). No fungal organisms were isolated from any cat. Potential pathogens were isolated significantly more often from CRS-affected cats than from control cats. Bacterial culture of biopsy specimens yielded aerobic bacteria (2/7 control cats and 4/10 CRS-affected cats) and anaerobic bacteria (2/10 CRS-affected cats). Although FHV-1 was not detected in nasal biopsy specimens from control or CRS-affected cats, FHV-1 DNA was detected via PCR assay in specimens from 4 of 7 control cats and 3 of 10 CRS-affected cats.

Conclusions and Clinical Relevance—Compared with findings in control cats, anaerobic bacteria, *Mycoplasma* spp, and a variety of potentially pathogenic organisms were detected more commonly in samples from cats with CRS. In both groups, FHV-1 was detected via PCR assay as a nonviable organism or in noncultivable amounts. (*J Am Vet Med Assoc* 2005;227:579–585)

Chronic nasal disease is an important problem in feline populations, and recent studies[1,2] have revealed that idiopathic **chronic rhinosinusitis (CRS)** is 1 of the 2 most common causes of sneezing and nasal discharge in cats. Although the etiology of sneezing and nasal discharge in cats is rarely determined in clinical practice, acute upper respiratory tract disease in kittens is commonly presumed to be caused by infection with **feline herpesvirus type 1 (FHV-1)**, feline calicivirus, *Chlamydophila felis*, or a combination of these agents. Of these organisms, FHV-1 is most commonly implicated in the chronic form of disease. Exposure to FHV-1 can be detected serologically in almost 100% of cats,[3] and 50% to 80% of exposed cats remain latent carriers of the virus.[4] In a large prospective study[5] involving privately owned cats, conjunctival swab specimens were assessed via **polymerase chain reaction (PCR)** assay and FHV-1 DNA was detected in 21% (98/462) of cats with surface ocular or upper respiratory tract disease (ie, conjunctivitis, keratitis, or rhinitis); however, FHV-1 DNA was detected in only 1% (1/95) of cats without clinical evidence of ocular or upper respiratory tract disease. In cats, experimental infection with FHV-1 may induce turbinate lysis,[6] a common feature of CRS, and it has been suggested that FHV-1 can remain latent within the nasal epithelium of cats.[7] Taken together, these data suggest that chronic or recurrent infection with FHV-1 contributes to the pathogenesis of CRS in cats.

Cats with CRS often have a transient response to antimicrobial treatment, and retrospective studies[1,8,9] have revealed that bacterial culture of various samples from affected cats often yields positive results. Michiels et al[1] reported that in 15 of 21 cats with nonspecific rhinitis, bacterial culture of nasal swabs (obtained by use of sterile technique) from most cats yielded growth of multiple bacteria; *Pseudomonas* spp (53% of samples) and *Escherichia coli* (40%) were isolated most frequently. In 1 study[8] of cats with chronic nasal discharge, *Pseudomonas* organisms were most commonly isolated from samples of material collected from the frontal sinus during surgery (7/11 cats), whereas results of another study[9] indicated that *Pasteurella multocida* was the most common isolate in nasal samples collected from cats with CRS (4/11). These studies differed with regard to sample collection methods, and the quantity of bacterial growth was not described.

The causes of chronic, recurrent nasal disease in adult cats have not been thoroughly investigated, and to the authors' knowledge, the interaction among potential infectious agents in cats with rhinitis has not been explored. In addition, the role of fungi in the development of CRS in cats has not been examined. The purpose of the study reported here was to determine detection rates for FHV-1, *Mycoplasma* spp, fungi, and bacteria in samples from the nasal cavities of cats with or without CRS. We hypothesized that FHV-1 infects and persists within or reactivates into the nasal epithelium of cats, thereby having a primary role in the

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Supported by grants from the Center for Companion Animal Health University of California, Davis (01-05-F), and the National Institutes of Health Heart, Lung, and Blood Institute (HL-03856).

Presented in part at the 21st Annual Forum of the American College of Veterinary Internal Medicine, Charlotte, NC, June 2003.

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pathogenesis of chronic upper respiratory tract disease of cats. We also hypothesized that bacteria or fungi may increase the severity of CRS by invading and overgrowing surface nasal epithelium. The study was designed to compare the detection of microorganisms in nasal epithelial samples from cats with CRS and control cats that lacked history or clinical signs of upper respiratory tract disease.

Materials and Methods

Study population—Ten cats with CRS were recruited from the patient population at the **University of California, Davis, Veterinary Medical Teaching Hospital (UCD-VMTH)**. All cats had a history of chronic mucopurulent nasal discharge (≥ 2 months' duration), sneezing, or stertorous breathing; these data were verified through discussion with the owners and from entries in the medical records. Eight of the 10 cats had previously been treated with systemically administered antimicrobials. Six of those cats received a penicillin-type drug PO for 7 to 10 days, and 4 were subsequently administered enrofloxacin PO (duration of treatment varied); 1 cat was treated with both drugs and was then administered doxycycline PO for 21 days. The antimicrobials that were administered to the remaining 2 cats were not known; prednisone had been prescribed for 1 of 10 CRS cats. Medications were discontinued at least 3 days prior to inclusion in the study. Cats with CRS underwent some or all tests included in a standard clinical assessment for this disease at our institution, including a CBC (n = 7), serum biochemical analyses (7), skull or dental radiography (2), computed tomography involving a helical high-speed scanner[a] (8) with 2-mm (1) or 3-mm (7) radiographic slices, and rostral rhinoscopy (10) involving a 2.8-mm rigid telescope[b] (8) or a 1.7-mm semiflexible endoscope[c] (2). For diagnostic imaging and rhinoscopy, each cat was premedicated and a bolus of propofol (6 mg/kg [2.73 mg/lb]) was administered IV; an endotracheal tube was placed, and anesthesia was maintained with isoflurane throughout the procedure. An injection of oxymorphone (0.05 mg/kg [0.02 mg/lb]) was given SC after the procedure for pain control.

Seven cats were included as a control group in this study; these cats were privately owned animals that had been euthanatized by use of an IV injection of an overdose of pentobarbital (as recommended in the 2000 Report of the AVMA Panel on Euthanasia) because of diseases unrelated to the respiratory tract and submitted for necropsy to the UCD-VMTH pathology laboratory. Four of the 7 control cats had previously been treated with antimicrobials (amoxicillin-clavulanic acid, cephalexin, doxycycline, and an unknown agent), and 1 cat had been treated with prednisone. Medications were discontinued at least 3 days prior to euthanasia and collection of samples. All control cats lacked historical evidence and clinical signs of upper respiratory tract disease. Immediately after euthanasia of the control cats, rhinoscopy was performed by use of a 1.7-mm semiflexible endoscope.[c]

In control cats and cats with CRS, both sides of the nasal cavity were assessed rhinoscopically and the degree of hyperemia, mucus accumulation, and turbinate destruction was scored as absent (score, 0), mild (1), moderate (2), or severe (3). Scores for these 3 characteristics were summed to provide a total rhinoscopic score for each nasal cavity.[10] Following rhinoscopy in each cat, a deep nasal flush was performed by use of an 8-F, sterile rubber catheter that was placed antegrade into the left side of the nasal cavity. To obtain the flush sample, the caudal region of the nasopharynx was obstructed via digital compression; 3 mL of sterile saline (0.9% NaCl) solution was infused via the catheter, and an aliquot was reaspirated. The sample was placed in semisolid anaerobic culture medium[d] for fungal culture, aerobic and anaerobic bacterial cultures, and mycoplasmal culture. After the nasal flush procedure was completed, nasal pinch biopsy specimens were obtained from the left and right sides of the nasal cavities by use of a 3-mm cup biopsy instrument.[e] In cats with CRS, notably diseased areas were targeted for biopsy rhinoscopically. In control cats, biopsy specimens were obtained without rhinoscopic guidance. Consent was obtained from owners of cats with CRS to collect 2 or 3 additional nasal biopsy specimens for use in this study. Two 3-pinch biopsy specimens were collected from each side of the nasal cavity in most control cats and cats with CRS; specimens were submitted for histologic or microbiologic assessment.

Histologic evaluation—Nasal biopsy specimens were immersion-fixed for 12 hours in neutral-buffered 10% formalin and embedded in paraffin; 4-µm-thick sections were cut and stained with H&E. All biopsy specimens were evaluated histologically in a masked fashion by a board-certified veterinary pathologist (HEVD) and were graded subjectively for the type and severity of inflammatory infiltrates and degree of turbinate remodeling.[10] Briefly, identification of a few inflammatory cells (approx < 20 cells) within the mucosa was considered normal. Severity of inflammation was assessed and graded as mild when a small number of inflammatory cells were diffusely present throughout the mucosa, moderate when notable numbers of inflammatory cells were diffusely present throughout the mucosa, and severe when the nasal mucosa was completely obscured by inflammatory cells. Proportions of lymphocytes, plasma cells, and neutrophils were assessed subjectively. On the basis of the predominant inflammatory cell type, 2 classifications of inflammation were made: neutrophilic or lymphoplasmacytic. Other inflammatory cells (macrophages, mast cells, eosinophils, and globular leukocytes) and epithelial ulceration were recorded if present. The degree of nasal turbinate remodeling was graded as mild, moderate, or marked.

Microbiologic assessments—Nasal flush samples and biopsy specimens from the left side of the nasal cavity of each cat were transported immediately to the UCD-VMTH microbiology laboratory and processed within 1 hour of collection. Flush samples were inoculated onto 5% sheep blood agar and MacConkey agar for isolation of aerobic bacteria, prereduced anaerobic *Brucella* plates[d] for anaerobic bacterial culture, a pleuropneumonia-like organism base with thallium acetate (antifungal agent) and penicillin G (antibacterial agent)[f] for culture of *Mycoplasma* spp, and inhibitory mold agar[g] for fungal culture. Prior to culture, biopsy specimens were placed in 1 mL of brain heart infusion on a sterile plate and macerated into fine fragments by use of a sterile No. 10 scalpel blade. Bacterial growth was assessed in a semiquantitative fashion by counting the number of quadrants with bacterial growth and was reported as 1+, 2+, 3+, or 4+. Standard biochemical methods were used to identify cultured bacteria.[11] Gram-negative bacilli that produced oxidase; did not ferment glucose, sucrose, or lactose; and did not produce indole, gas, or H_2S but that were not classified as *Bordetella bronchiseptica, Pseudomonas* spp, or *Acinetobacter* spp were described as nonenteric gram-negative rods. The following microbial species detected in nasal samples were considered potentially pathogenic: *P multocida*, *Streptococcus viridans*, *B bronchiseptica*, *Staphylococcus intermedius, Pseudomonas aeruginosa, E coli, Corynebacterium* spp, *Actinomyces* spp, *Mycoplasma* spp, and all anaerobic species.[11] Coagulase-negative *Staphylococcus* spp, nonenteric gram-negative rods, and *Flavobacteria* spp were considered nonpathogenic. Any isolate of *B bronchiseptica* was characterized via pulsed gel electrophoresis to rule out a vaccinal strain of the organism.

One nasal biopsy specimen from the right side of the nasal cavity of each cat was placed in 2 mL of sterile medium

(1% Dulbecco modified Eagle medium containing 5% fetal calf serum, 50 µg of gentamicin/mL, 2.5 µg of amphotericin B/mL, and 100 µg/mL of penicillin G potassium) for virus isolation and stored at –20°C prior to culture. Subsequently, each biopsy specimen was thawed and homogenized on ice in a sterile glass tissue homogenizer. Aliquots (2 mL) of tissue homogenate propagated at 37°C on Crandell-Rees feline kidney cells were used for virus isolation. Starting 12 to 20 hours after inoculation, cultures were inspected for cytopathic effects typical of FHV-1 and feline calicivirus every 24 hours for as long as 10 days.

A second biopsy specimen from the right side of the nasal cavity of each cat was placed in sterile phosphate-buffered saline solution and kept at –20°C until DNA extraction was performed by use of a commercially available kit[h] containing proteinase K for tissue lysis. Extracted DNA was eluted into Tris-EDTA buffer and stored at –20°C until the PCR assay was performed. The DNA was quantified by use of a spectrophotometer, and approximately 300 ng of DNA was used in each FHV-1 PCR reaction. The PCR protocol targeted a 322-base pair region of the FHV-1 thymidine kinase gene.[12] The PCR reaction conditions involved 40 cycles of amplification of target DNA and a final 10-minute extension step. The reaction product was visualized following electrophoresis on an agarose gel and staining with ethidium bromide. Under these conditions, this technique reliably detects ≥ 240 copies of FHV-1 template DNA.[12]

Statistical analyses—Age and sex distributions were compared between groups by use of Student *t* tests. For each microorganism, detection rate was defined as the number of samples in which that organism was identified. In nasal flush samples, bacterial (aerobic, anaerobic, and mycoplasmal) detection rates were assessed. In biopsy specimens, detection rates were calculated for bacteria, viable FHV-1 (data from virus isolation procedures performed on biopsy specimens from the right side of the nasal cavity), and FHV-1 DNA (data from PCR assays performed on biopsy specimens from the right side of the nasal cavity). Detection rates of bacteria in nasal flush samples and biopsy specimens were recalculated for the organisms that were considered potentially pathogenic, and mean detection rates were calculated for pathogens. Biopsy specimens were also assessed for viral agents; therefore, statistical analysis of biopsy specimen pathogen data included results of FHV-1 PCR assay with results of viral isolation of potentially pathogenic organisms.

To compare sampling techniques, detection rates for all microorganisms and those considered potentially pathogenic in nasal flush samples and biopsy specimens from CRS-affected and control cats were compared by use of a Student *t* test. The hypothesis that the nasal flush samples or biopsy specimens obtained from cats with CRS had higher detection rates for bacteria and total pathogens than those obtained from control cats was assessed by use of a Student *t* test. Detection rates for FHV-1 were compared between control cats and cats with CRS by use of the κ statistic, and significance was assessed by use of a Fisher exact test. The association between detection rates for *Mycoplasma* spp and CRS was assessed by use of a Fisher exact test. In all analyses, a value of $P \leq 0.05$ was considered significant.

Results

Study population—Seven cats met the entry criteria for use as control cats, and 10 client-owned cats with histories and clinical signs consistent with CRS were designated as CRS-affected cats (**Table 1**). Cats with CRS were 0.5 to 20 years old, and control cats

Table 1—Clinical and diagnostic imaging data from 10 cats with chronic rhinosinusitis (CRS).

Cat (No.)	Age (m)	Duration of nasal discharge (m)	Ocular discharge	Rhinoscopy score (L, R)	Findings of diagnostic imaging procedures*
1	17	> 2 (L = R)	Purulent (bilateral)	9, 9	Increased soft tissue opacity in the nasal cavity (R > L). Frontal sinuses diffusely opacified.
2	7	6 (R > L)	Purulent (bilateral)	7, 9	Bilateral nasal passage opacification and nasal turbinate destruction. Bilateral frontal sinus opacification (L > R).
3	96	4 (R)	Absent	3, 5	Moderate turbinate destruction and soft tissue opacification. Moderate fluid in the right frontal sinus
4	24	12 (R)	Absent	5, 8	Patchy increase in soft tissue opacity within the nasal cavities bilaterally. Soft tissue density filling the right sphenopalatine sinus.
5	Adult	6 (R)	Absent	3, 4	Diffuse loss of fine turbinate structures with a cavitary appearance (R). Irregular soft tissue structure in the right frontal sinus. Mild thickening of the mucosa of the left nasal turbinates.
6	11	5 (L = R)	Serous (left eye)	5, 4	Moderate patchy soft tissue opacification of the nasal cavity and paranasal sinuses bilaterally. Turbinate destruction most notable rostrally. Fluid opacification of frontal sinus (L > R).
7	88	7 (R)	Purulent (left eye)	4, 4	Soft tissue opacity of both nasal cavities (R > L) and involving both frontal sinuses and the right sphenopalatine sinus. Turbinate destruction bilaterally (R > L). Osteolysis and extensive bony remodeling.
8	240	24 (L = R)	Absent	6, 4	Moderate soft tissue opacification of much of the ventral portion of the nasal cavity from the nares to the cribriform plate.
9	27	18 (L = R)	Absent	7, 7	Patchy soft tissue opacification of the nasal cavities bilaterally and left front sinus, with complete opacification of the right sphenopalatine sinus. Moderate maxillary turbinate loss bilaterally.
10	180	5 (L > R)	Serous (left eye)	3, 3	Moderate fluid accumulation ventrally in the midportion of the nasal passages bilaterally.

*Skull radiography performed in cats 1 and 2 and computed tomography in the remaining cats.
L = Left side of the nasal cavity. R = Right side of the nasal cavity.

were 0.75 to 18 years old. Of the cats, 3 in the CRS-affected group and 1 in the control group were purebred. Females predominated in both the CRS-affected and control groups (6/10 and 6/7 cats, respectively). All cats except 1 control and 1 CRS-affected cat were neutered. No significant ($P = 0.20$) differences in age or sex were detected between the CRS-affected and control groups. Median duration of clinical signs prior to referral for cats with CRS was 6 months (range, 3 to 24 months). Five cats with CRS had purulent (n = 3) or serous (2) ocular discharge, but keratoconjunctivitis was not detected in any cat. All cats with CRS were otherwise healthy.

Results of the CBCs and serum biochemical analyses performed prior to anesthesia indicated mild abnormalities in 7 of 7 cats with CRS (CBCs were not performed for the other 3 cats). These abnormalities included mild leukocytosis (n = 3), basophilia (2), eosinophilia (1), thrombocytosis (1), and hyperglobulinemia (1). In 2 cats with CRS, skull or dental radiography revealed opacities indicative of soft tissue or fluid in the nasal cavity and both frontal sinuses (Table 1). In 8 CRS-affected cats for which computed tomography was performed, findings included soft tissue opacification of the rostral nasal cavity (n = 8), frontal sinus (5), or sphenopalatine sinus (3). In 2 cats, both the frontal and sphenopalatine sinuses were opacified. Computed tomography revealed turbinate destruction in 5 of the 8 cats and lysis of nasal and frontal bones in 1 cat (cat 7). The cribriform plate was intact in all CRS-affected cats but was asymmetric in 1 cat (cat 3).

In control cats, rhinoscopy revealed minimal hyperemia or mucus accumulation and no turbinate destruction; therefore, the overall rhinoscopic score for both nasal cavities in all control cats was zero. By contrast, all cats with CRS had evidence of mild, moderate, or severe hyperemia and mucoid discharge in both nasal cavities. In 3 CRS-affected cats, mucoid discharge was more severe from the right side of the nasal cavity than from the left. Turbinate destruction was detected rhinoscopically in all cats with CRS but varied in severity among cats and between sides of the nasal cavity. Overall, 4 cats had moderate to severe bilateral turbinate destruction, 3 cats had primarily left-sided destruction, 2 cats had moderate right-sided destruction, and 1 cat had mild bilateral destruction. The median total rhinoscopic score for these cats was 5.5 (range, 3 to 9).

Histologic findings—Nasal biopsy specimens were obtained from all cats; in 2 of 7 control cats and 7 of 10 cats with CRS, nasal biopsy specimens obtained from both the right and left sides of the nasal cavity were evaluated (**Table 2**). Among the control cats, nasal inflammation was absent in 2, minimal in 4, and moderate in 1 that had squamous cell carcinoma of the tongue base. One cat with CRS (cat 10) had no histologic lesions in the left nasal cavity and mild neutrophilic inflammation in the right. The remaining cats with CRS had moderate to severe neutrophilic (n = 8) or lymphoplasmacytic (1) rhinitis. Also, intralesional bacteria were detected in 2 cats with CRS, ulceration of the epithelium was detected in 2 cats, and turbinate destruction and remodeling were detected in 6 cats. In 7 affected cats from which nasal biopsy specimens were collected bilaterally, 3 had primarily unilateral histologic lesions.

As previously reported,[10] rhinoscopic lesions did not always reflect the severity of histopathologic changes, although rhinoscopic scores were low in all control cats. Overall, the data indicated that clinical signs and findings of imaging procedures and histologic evaluation did not correspond in all cats (Tables 1 and 2).

Microbiologic assessments—Results of microbial cultures, virus isolation procedures, and PCR assay were obtained for CRS-affected and control cats (Table 2). When all data (ie, detected organisms [bacteria, mycoplasma, fungi, and viruses], findings in right and left sides of the nasal cavities, and results of all detection techniques for all cats [control and CRS-affected]) were considered simultaneously, microorganisms were detected in the nasal cavity of all but 1 cat (cat 12). This individual was a control cat in which no evidence of inflammation was detected histologically. Overall detection rates for all bacteria did not differ ($P = 0.08$) between nasal flush samples (13/17 samples) and biopsy specimens (8/17 specimens). Detection rates for potential bacterial pathogens also did not differ ($P = 0.20$) between nasal flush samples (9/17 samples) and biopsy specimens (7/17 specimens).

Mean total bacteria detection rates for nasal flush samples did not differ significantly between cats with CRS and control cats. In control cats, aerobic bacteria were cultured from 5 of 7 flush samples; however, a potentially pathogenic bacterium (*S viridans*) was isolated from only 1 (cat 13). Cultures for *Mycoplasma* spp and anaerobic bacteria yielded negative results for samples from all control cats. Therefore, when opportunistic organisms were removed from the analysis, potentially pathogenic bacterial organisms were isolated from nasal flush samples obtained from cats with CRS (8/10 cats) significantly ($P = 0.03$) more often than they were isolated from samples obtained from control cats (1/6).

Culture of nasal flush samples from cats with CRS yielded aerobic bacteria (n = 9), *Mycoplasma* spp (2), and anaerobic bacteria (3; Table 1). Potentially pathogenic organisms were isolated in culture of samples from 8 of 9 CRS cats. In the cat in which *B bronchiseptica* was identified, pulsed gel electrophoresis revealed that the organism had a molecular identity that corresponded with that of a vaccinial strain of *B bronchiseptica*, and the cat was known to have received an intranasal *Bordetella* vaccine; this pulsed gel electrophoresis technique, including data from this cat, has been previously published.[13] Anaerobic bacterial culture yielded positive results in 3 cats with CRS, and *Bacteroides* spp were the most common isolates. No fungal organisms were isolated in cats with CRS. A single colony of fungus (*Bergeyella zoohelcum*) was cultured from the flush specimen of 1 control cat and was considered a contaminant. Nasal flush samples from 2 cats with CRS yielded positive results for *Mycoplasma* spp; no mycoplasmal growth was obtained via culture of samples from control cats. No mycoplasmal growth

Table 2—Results of microbiologic and histologic evaluations of nasal flush samples and biopsy specimens collected from 10 cats with CRS and 7 control cats.

Cat No.	Bacterial culture results: Flush sample	Bacterial culture results: Biopsy specimen	FHV-1 virus isolation	FHV-1 PCR assay	Histologic findings
CRS-affected					
1	*_Pasteurella multocida_ 1+ Coagulase-negative _Staphylococcus_ 1+	*_P multocida_ 1+	Neg	Neg	Severe, chronic, diffuse neutrophilic rhinitis with intralesional bacteria (L and R)
2	*_Escherichia coli_ 1+ *_Peptostreptococcus anaerobius_ 1+ *_Bacteroides fragilis_ 1+	No growth	Neg	Neg	Moderate neutrophilic, ulcerative rhinitis (L)
3	Nonenteric gram-negative rods 1+ *_Bacteroides_ or _Prevotella_ 4+ *_Fusobacterium nucleatum_ 4+ *_P anaerobius_ 4+	No growth	Neg	Pos	Moderate lymphoplasmacytic rhinitis with focal ulceration and marked turbinate remodeling (R)
4	Nonenteric gram-negative rods 1+ Coagulase-negative _Staphylococcus_ 1+	*_Streptomyces_ 1+	Neg	Pos	Moderate neutrophilic, ulcerative rhinitis (L)
5	*_Corynebacterium ulcerans_ 1+	*_C ulcerans_ 3+ Coagulase-negative _Staphylococcus_ 3+	Neg	Neg	Mild neutrophilic rhinitis (L) Severe neutrophilic rhinitis with mild turbinate remodeling (R)
6	*_P multocida_ 2+ *_Bordetella bronchiseptica_ 2+ *_Streptococcus viridans_ 2+ *Nonfermenter group 3 2+ *_Mycoplasma felis_ 4+	*_Proprionibacterium acnes_ 1+	Neg	Neg	Severe neutrophilic rhinitis (L and R) with marked (L) and mild (R) turbinate remodeling
7	*_Pseudomonas aeruginosa_ 2+	*_P aeruginosa_ 1+	Neg	Neg	Moderate neutrophilic and ulcerative rhinitis with mild turbinate remodeling (L) Mild lymphocytic rhinitis (R)
8	*_Actinomyces slackii_ 1+ *_B ureolyticus_ 3+	*_B ureolyticus_ 1+	Neg	Neg	Severe neutrophilic and lymphoid rhinitis with intralesional bacteria (L and R) with mild (L) and moderate (R) turbinate remodeling
9	No growth	No growth	Neg	Pos	Moderate neutrophilic and lymphoplasmacytic rhinitis with moderate turbinate remodeling (L and R)
10	*_P multocida_ 1+ *_M felis_ 1+	No growth	Neg	Neg	No histologic lesions (L) Mild neutrophilic rhinitis (R)
Control					
11	No growth	No growth	Neg	Pos	Within normal limits (L)
12	No growth	No growth	Neg	Neg	Within normal limits (L)
13	Coagulase-negative _Staphylococcus_ 3+ *_Streptococcus viridans_ 3+ Nonenteric gram-negative rods 1+	Coagulase-negative _Staphylococcus_ 3+ *_S viridans_ 3+ Nonenteric gram-negative rods 1+	Neg	Pos	Mild acute neutrophilic rhinitis (R)
14	Nonenteric gram-negative rods 1+	No growth	Neg	Pos	Mild subacute neutrophilic and lymphocytic rhinitis (L)
15	Coagulase-negative _Staphylococcus_ 2+	Coagulase-negative _Staphylococcus_ 2+	Neg	Neg	Focal mild neutrophilic rhinitis (L)
16	Coagulase-negative _Staphylococcus_ 2+	No growth	Neg	Pos	Moderate diffuse lymphocytic rhinitis (L and R)
17	†_Bergeyella zoohelcum_ 1+	No growth	Neg	Neg	Minimal to mild lymphocytic and neutrophilic rhinitis (L and R)

*Potentially pathogenic bacteria. †Fungal contaminant.
FHV-1 = Feline herpesvirus type 1. PCR = Polymerase chain reaction. Neg = Negative. Pos = Positive.
See Table 1 for remainder of key.

was detected via culture of nasal biopsy specimens from any cat. Regardless of the sampling technique, differences in detection rates for *Mycoplasma* spp between cats with CRS and control cats were not significant (nasal flush samples, $P = 0.47$; biopsy specimens, $P = 1.00$), although very small numbers could be assessed.

Feline herpesvirus type 1 was not isolated from any biopsy specimen, although FHV-1 DNA was detected via PCR assay in nasal biopsy specimens obtained from 3 cats with CRS and 4 control cats. There was no significant ($P = 0.35$) difference in detection of FHV-1 in cats with CRS, compared with control cats. With regard to all potential bacterial pathogens and FHV-1, there was no significant ($P = 0.22$) difference between detection rates in nasal biopsy specimens from cats with CRS and control cats.

Discussion

The present prospective controlled study was intended to investigate the relative roles of bacteria, fungi, *Mycoplasma* spp, and FHV-1 in the etiopathogenesis of chronic upper respiratory tract disease in cats. Our data indicated that potentially pathogenic bacteria were isolated more commonly from cats with CRS than from cats without signs of respiratory tract disease; however, the rates of detection of FHV-1 DNA in nasal biopsy specimens from control and CRS-affected cats were not significantly different. Subjectively, compared with findings in control cats, a greater number and wider variety of bacterial species were detected in cats with CRS; *Mycoplasma* spp and anaerobic bacteria were isolated only from cats with CRS. The isolation of these various organisms is of clinical and therapeutic importance because susceptibility to antimicrobials varies substantially among bacterial types. In addition, detection of bacterial organisms in biopsy specimens could be suggestive of a more established infection (including infection of submucosal tissues) than colonization of the epithelial surface only. Whether bacterial species are causally related to disease or accumulate secondary to nasal inflammation and damage could not be determined in the present

study. Also, the clinical relevance of different detection rates of organisms via evaluation of nasal flush samples versus biopsy specimens is challenging to resolve. For 5 of 9 CRS-affected cats, a particular bacterial organism identified in nasal flush samples was also detected in biopsy specimens; other bacterial species were also isolated from the nasal flush samples obtained from these cats. However, for 3 CRS-affected cats, microorganisms were detected in nasal flush samples but not in biopsy specimens. This could be attributed to surface bacterial infection or colonization. Alternatively, it may reflect loss or death of organisms during the additional tissue handling required for processing of biopsy specimens. Interestingly, for 2 CRS-affected cats, an organism that was cultured from biopsy specimens was not detected in the corresponding nasal flush samples, and the bacterial species detected in the nasal flush samples were not cultured from the biopsy specimens. Thus, to thoroughly evaluate the bacterial component of rhinitis in cats with CRS, it is worth considering culture of both nasal flush samples and biopsy specimens.

The involvement of various infectious pathogens in development of acute upper respiratory tract disease in cats has been examined in many clinical and experimental studies.[3,5,14-16] Most studies[3,5] of naturally occurring upper respiratory tract disease in cats have focused on isolation of a single etiologic agent, although to our knowledge, an analysis of the interrelationship among microbial agents has not been previously performed. In the present study, a role for active viral infection in naturally occurring CRS of cats could not be established, although a plethora of bacterial organisms were isolated. Experimental challenge studies in cats are required to determine whether bacteria are primarily responsible for ongoing disease or are present because of accumulation in diseased tissue.

Primary bacterial pathogens of the nasal cavity in cats, such as *B bronchiseptica*, have been identified.[17] In our study, *B bronchiseptica* was isolated from 1 cat with chronic nasal disease; however, molecular typing revealed that the organism was present probably as a result of retention in the nasal cavity of a vaccinal strain of *Bordetella*. Whether this organism initiated pathologic changes associated with chronic nasal disease or could not be eliminated from the nasal cavity because of preexisting disease remains unknown. In cats, *Mycoplasma* spp have also been identified as primary pathogens of the lower respiratory tract[18]; however, in the present study, both *B bronchiseptica* and mycoplasmal organisms were isolated from nasal cavities in association with other bacteria. It is intriguing to speculate about a potential role for *P multocida* in perpetuating nasal disease in cats. *Pasteurella multocida* is considered a commensal of the upper respiratory and digestive tracts of cats; however, a toxigenic strain of this organism has been implicated in the development of nasal turbinate atrophy in pigs,[19,20] and turbinate destruction is a prominent finding in cats with chronic nasal disease.

Although bacteria can be cultured from samples of the nasal discharge of cats with chronic rhinitis,[2,9] cats with rhinitis often have only a transient response to antimicrobial treatments and recurrences of rhinitis are common. This might suggest that the role of bacteria is secondary to the overall pathogenesis of the disease. However, it is also possible that inappropriate antimicrobials are chosen or used for an insufficient treatment period in cats with well-established bacterial infection. In the present study, potentially pathogenic bacteria were isolated from nasal biopsy specimens from 6 of 10 cats with CRS, suggestive of tissue infection with bacteria. By contrast, such potential pathogens were isolated from a deep epithelial sample from only 1 control cat. This difference between the 2 groups was not significant but might be considered of some clinical relevance.

Virus isolation, immunofluorescent antibody assays, and serum neutralizing and ELISA antibody titers have been used to confirm exposure to or presence of FHV-1 in cats with upper respiratory tract disease, but the correlation between test results and disease state is low.[3] Additionally, most studies have involved serologic evaluations,[3] identification of pathogens in the conjunctiva,[3] or culture of organisms from the oropharynx[14-16] rather than direct assessment of nasal tissue specimens. In the study of this report, virus isolation procedures in control cats and cats with CRS failed to yield growth of FHV-1, although viral DNA was detected via PCR assay in some cats in both groups. Several methodological issues may have contributed to the failure to isolate FHV-1 in culture or to detect FHV-1 DNA in more cats via PCR assay. Nasal flush samples were not assessed for viral presence by either culture or PCR assay. The PCR assay used in the present study has a relative detection rate of only 43%, compared with that of other PCR protocols[21]; furthermore, evaluation via PCR assay of ocular samples obtained from both eyes of a cat may yield positive results for FHV-1 in only 1 eye.[22] Although it is possible that collection of nasal flush samples bilaterally for FHV-1 PCR assay or use of a different PCR technique would have resulted in a higher number of cats in which FHV-1 DNA was detected, it is unlikely this would have resulted in significant differences between CRS-affected and control groups in the present study.

In a retrospective study[2] of cats with nasal disease, FHV-1 was isolated from nasal or oropharyngeal swabs of 2 of 11 cats. In the present investigation, actively replicating virus was not detected in cats with or without nasal disease, but viral DNA was detected in a similar number of cats in each group. It remains possible that the underlying pathogenesis of nasal damage is initiated by FHV-1–mediated cytolysis or induced by virally directed, immune-mediated destruction and perpetuation of inflammation in the absence of ongoing viral infection. Thus, FHV-1 could be the initial antigenic stimulus for development of chronic immune-mediated pathologic changes in cats' noses, similar to the role proposed for the virus in the development of herpetic stromal keratitis in cats.[23] Onset of clinical signs of nasal disease could be initiated by permanent destruction of nasal structures following severe acute viral cytolysis.[6] Loss of normal nasal architecture disrupts mucociliary function and could result in trapping of mucus and bacteria within the nasal cav-

ity, thereby creating a favorable environment for bacterial colonization. Given the lack of detection of viable FHV-1 in the cats of the present study, it is unlikely that the virus is reactivated from the trigeminal ganglia into nasal tissues to cause cumulative cytolytic destruction of the nasal epithelium.

At present, treatment of chronic rhinitis relies on administration of antimicrobials against presumptive secondary bacterial infection. Results of aerobic and anaerobic bacterial culture and mycoplasmal culture of deep nasal flush samples or nasal tissue should be considered to guide antimicrobial selection for individual cats. Findings of our study failed to confirm a role for active viral infection in the pathogenesis of CRS in cats, and the detection of FHV-1 DNA in both CRS-affected and control cats further obscures the potential importance of this virus in clinical disease. It is possible that FHV-1 initiates nasal disease in certain cats, perhaps as a result of severe infection at an early age or specific immunologic responses. Longitudinal studies of kittens infected with FHV-1 early in life or experimental investigations in cats may provide further information on the role of FHV-1 in chronic nasal disease of cats.

a. General Electric Medical Instruments, Milwaukee, Wis.
b. Richard Wolf Medical Instruments Corp, Vernon Hills, Ill.
c. MDS Inc, Brandon, Fla.
d. Anaerobe Systems, Morgan Hill, Calif.
e. Karl Storz Veterinary Endoscopy, Goleta, Calif.
f. UC Davis media room, Davis, Calif.
g. Hardy Diagnostics, Santa Maria, Calif.
h. Qiagen DNeasy tissue kit, Qiagen Inc, Valencia, Calif.

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