*Journal of Feline Medicine and Surgery* (2008) **10**, 551–557
doi:10.1016/j.jfms.2008.04.002

ELSEVIER

jfms

# Prevalence of selected infectious organisms and comparison of two anatomic sampling sites in shelter cats with upper respiratory tract disease

**Julia K Veir** DVM, PhD, Diplomate ACVIM*, **Rebecca Ruch-Gallie** DVM, MS, **Miranda E Spindel** DVM, **Michael R Lappin** DVM, PhD, Diplomate ACVIM

*College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Campus Delivery 1620, Fort Collins, CO 80523, United States*

In order to describe the isolation rates of potential pathogens and to compare anatomic sampling site suitability, nasal and pharyngeal swabs were taken from cats with acute clinical upper respiratory disease in a humane society. DNA of feline herpesvirus-1 was amplified from 51 of 52 cats sampled, *Mycoplasma* species were cultured or detected by PCR in samples from 34 of 42 cats sampled for both culture and PCR, and *Bordetella bronchiseptica* was isolated from three of 59 cats sampled for aerobic culture. A single swab was positive for calicivirus and no swabs were positive for *Chlamydophila felis*. *Mycoplasma*, *Pasteurella* and *Moraxella* species were all isolated from at least one cat in which no primary pathogen was identified. With the exception of *B. bronchiseptica*, which was detected in nasal swabs only, recovery rates for all suspect primary pathogens were comparable between sampling sites.

*Date accepted: 1 April 2008*



Upper respiratory tract disease (URTD) continues to be a major problem in shelters and humane societies; many report it to be a leading cause of euthanasia in cats in traditional shelters (Foley and Bannasch 2004) and a major financial burden for no-kill shelters (Pedersen et al 2004). Multiple organisms have been isolated from cats with acute upper respiratory tract disease (URTD) including feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV), as well as many aerobic and anaerobic bacteria (Cape 1992, Helps et al 2005). With the exception of *Bordetella bronchiseptica* and *Chlamydophila felis*, most bacterial infections are thought to be secondary to other primary diseases. Although historically implicated in feline lower respiratory tract disease (Padrid et al 1991, Foster et al 1998, 2004), *Mycoplasma* species have recently been proposed to be associated with chronic (Johnson et al 2005) and acute (Bannasch and Foley 2005) feline URTD.

Microbial detection techniques utilized in URTD prevalence studies include aerobic culture, anaerobic culture, virus isolation, polymerase chain reaction (PCR) assay for microbial DNA, and reverse transcriptase PCR (RT-PCR) assay for RNA viruses like FCV. However, comparison of results amongst previous prevalence studies cannot be made because of lack of standardization in microbial detection techniques and sampling sites. In order to maximize limited diagnostic and treatment resources, shelter personnel must know not only the most common organisms associated with URTD, but also the optimal sampling site and diagnostic assays for use in refractory or sentinel cases. Therefore, the objectives of this study were to determine the prevalence rates of aerobic bacteria, select viruses, and *Mycoplasma* species in clinically ill cats residing in a humane society by use of standardized methods of microbial isolation applied to samples collected from two separate but commonly referenced sampling sites (nasal and oropharyngeal).

*Corresponding author. E-mail: jveir@colostate.edu

1098-612X/08/060551+07 $34.00/0 

## Materials and methods

### Experimental design

Samples were collected from cats residing in a combined rural and urban humane society shelter in north central Colorado between January and December 2003. Sixty-one cats with clinical signs of URTD (oculonasal discharge, sneezing, and stertorous breathing) with a qualifying clinical score were entered concurrently into the study described here as well as an antibiotic therapy comparison study (Ruch-Gallie et al in press). While vaccination records are not available, the policy of this humane society is to administer a modified live feline viral rhinotracheitis-calicipanleukopenia (FVRCP) vaccine on admission and so it is possible some cats had been recently vaccinated. Within 24 h of admission, three swabs from both the nasal cavity and oropharynx were taken from each cat prior to treatment. Nasal swabs were obtained by gently rolling a sterile transurethral culture swab (Ca alginate swabs, Ultrafine Al, Fischer Scientific) in the anterior aspect of the right nares after removing any excess mucus. Oropharyngeal swabs were obtained using a sterile cotton tipped applicator gently rotated in the oropharynx of each cat. One swab from each sampling site was placed into a commercial transport medium (BBL CultureSwab Plus Amies Medium with Charcoal, Becton, Dickinson and Company; Sparks, MD) and submitted for culture of *Mycoplasma* species and aerobic bacteria within 4 h. A second swab was placed in viral transport medium (viral transport media: modified Eagle's medium (MEM) with 1% HEPES (*N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid), 4% bovine growth serum, 0.0025% amphotericin B + antibiotics) and submitted for virus isolation within 4 h. The final swab was placed in 1 ml sterile phosphate buffered saline solution (PBS), allowed to equilibrate for 2−3 h at room temperature according to the DNA extraction kit (Qiagen, Valencia, CA) manufacturer's instructions and then stored at −70°C until assayed for the presence of FHV-1 DNA, *Chlamydophila felis* DNA, *Mycoplasma* species DNA, and FCV RNA.

### Aerobic bacteria and Mycoplasma species culture

Discharge collected on one oropharyngeal and nasal swab from each cat was inoculated on to blood agar, MacConkey, Columbia agar and Friis broth agar plates, then placed in 2 ml of Friis broth and incubated at 37°C with 5% $CO_2$ for 24 h. One hundred microliters of the resulting liquid culture was again inoculated on to Friis broth agar plates. All plates were incubated at 37°C with room air (blood agar, MacConkey), 5% $CO_2$ (Friis broth) or 10% $CO_2$ (Columbia) for 72 h immediately after inoculation. Aerobic bacteria were characterized using standard biochemical methods. *Mycoplasma* species cultures were considered positive if colonies had a microscopic 'fried egg' appearance on selective agar (Friis broth).

### Virus isolation

After low speed centrifugation at 4°C, sample supernatant (~1 ml) was filtered using a sterile 0.45 μm filter and inoculated in triplicate on to a monolayer of Crandall Rees feline kidney (CRFK) cells. After an adsorption period of 1−2 h at 37°C in 5% $CO_2$, the sample was decanted and cells were incubated with 1 ml of MEM with 3% fetal bovine serum (FBS) in the same environment for a maximum of 7 days. Cells were checked daily for evidence of cytopathic effect after comparison to a control well of naïve CRFK cells. Samples that did not show evidence of cytopathic effect after 7 days were passaged a second time as previously described before being declared negative. For positive samples, the virus causing cytopathic effects was assumed to be either FHV-1 or FCV based on the results of the FHV-1 PCR assay and FCV RT-PCR assay performed on the same sample.

### PCR and RT-PCR assays

Detection of FHV-1, *Mycoplasma* species, and *Chlamydophila felis* DNA in swabs was performed using previously published assays (Sykes et al 1997, Baird et al 1999, Burgesser et al 1999) after extraction of DNA from one-half the volume of the sample using a commercially available kit (DNeasy kit, Qiagen, Valencia, CA) according to manufacturer's protocols. RNA was extracted from the remainder of the sample using a commercially available kit (RNeasy kit, Qiagen, Valencia, CA) and the RNA was assessed using two previously published RT-PCR assays for the amplification of FCV (Radford et al 1997, Sykes et al 1998). Controls for nucleic acid amplification techniques included extraction of an equivalent volume of PCR grade water during nucleic acid extraction and a negative control consisting of PCR grade water in place of template and a positive control consisting of nucleic acid extracted from positive viral cultures during amplification.

### Statistical analyses

Concordance of assay and sampling site results for FHV-1, FCV, *C felis*, *B bronchiseptica*, and *Mycoplasma* species were evaluated using the kappa statistic with indices of specific agreement ($p_{pos}$ and $p_{neg}$) (Feinstein 2002). Definitions of quantitative significance for kappa were as defined by Landis and Koch (1977). Any analysis that required larger than a $2 \times 2$ agreement matrix (ie, comparison of agreement between results for two organisms from both sampling sites) and concordance of assay and sampling site results for the remaining detected organisms were evaluated using a simple proportion of agreement (Feinstein 2002).

## Results

Overall, 65 cats were enrolled in the study. Due to assay technical difficulties, some assays had to be repeated on a subset of samples. Therefore, due to sample volume limitations, not all assays were run on all samples. The number of cats tested is detailed within the results section for the assay for each organism.

**Table 1**. Aerobic organisms isolated from nasal or pharyngeal swabs of cats with URTD

| | Aerobic culture ($n = 59$) $N$ (% positive) | |
|---|---|---|
| | Nasal | Pharyngeal |
| *Bordetella bronchiseptica* | 3 (5.1) | 0 (0) |
| *Mycoplasma* species | 28 (47.5) | 31 (52.5) |
| *Pasteurella* species | 19 (32.2) | 43 (72.9) |
| *Moraxella* species | 4 (6.8) | 21 (35.6) |
| Coagulase (−) *Staphylococcus* species | 4 (6.8) | 6 (10.2) |
| Non-hemolytic *Streptococcus* species | 4 (6.8) | 7 (11.9) |
| *Flavobacterium* | 0 (0) | 10 (17.0) |
| *Staphylococcus aureus* | 0 (0) | 2 (3.4) |
| *Staphylococcus intermedius* | 3 (5.1) | 0 (0) |
| *Enterobacter* species | 0 (0) | 1 (1.7) |
| *Escherichia coli* | 0 (0) | 1 (1.7) |
| *Streptococcus* β-hemolytic | 7 (11.9) | 6 (10.2) |
| *Cornebacterium* species | 1 (1.7) | 1 (1.7) |
| *Haemophilus* species | 0 (0) | 1 (1.7) |
| *Actinomyces* species | 2 (3.4) | 1 (1.7) |
| *Micrococcus* species | 1 (1.7) | 0 (0) |

Within 24 h of admission, samples were collected from admissions to a combined rural and urban humane society shelter with clinical signs of URTD. Routine aerobic culture was performed on samples within 4 h of collection.

### Comparison of sampling sites for microbial recovery

Aerobic bacteria were cultured from the majority of cats regardless of sampling site (nasal: 45/59, 76.3%; pharyngeal: 56/59, 94.9%, Table 1). However, aerobic bacterial culture results from nasal and pharyngeal swabs were in complete agreement for only seven of 59 cats sampled. Suspected primary pathogens (*C felis*, *B bronchiseptica*, *Mycoplasma* species, FCV, and FHV-1) were cultured or amplified from a variable number of cats and results differed between sampling sites depending on the organism and assay (Table 2). RNA of FCV was not amplified from any sample using the first RT-PCR assay (Sykes et al 1998) we used, prompting the evaluation of the samples in a second assay (Radford et al 1997). In the second RT-PCR assay, FCV was detected in a single sample. In addition, *C felis* was not amplified from any sample assayed. Because of the low prevalence rates of FCV and *C felis*, analysis of cumulative primary pathogen results between sampling sites were compared only for FHV-1, *Mycoplasma* species and *B bronchiseptica* (Table 2). For 39 cats, PCR assay results for both FHV-1 and *Mycoplasma* species from both sites were available; 35 of 39 (89.7%) nasal swabs and 37 of 39 (94.9%) pharyngeal swabs were positive for one or both of the organisms. Results were in agreement for the two sampling sites for both organisms via PCR assay in 19 of 39 (48.7%) cats. Culture for FHV-1 (virus isolation), *Mycoplasma* species and *B bronchiseptica* yielded positive results for at least one of the organisms in 39 of 51 (76.5%) in both nasal and pharyngeal samples. Agreement between the two sampling sites for all three organisms by microbiologic culture was 62.7% (32 of 51 cats, Table 2).

### Comparison of PCR assay and culture for detection of Mycoplasma species

Results of both PCR assay and culture for *Mycoplasma* species were available for 42 cats. Of the 42 nasal samples, 26 (61.9%) were positive as determined by PCR assay and 21 (50.0%) were positive via bacteriologic culture ($\kappa = 0.67$, $p_{pos} = 0.85$, $p_{neg} = 0.81$). Of the 42 pharyngeal samples, 29 (69.0%) were positive by PCR assay and 23 (54.8%) were positive on bacteriologic culture ($\kappa = 0.41$, $p_{pos} = 0.77$, $p_{neg} = 0.63$). When

**Table 2**. Concordance of results of detection from nasal and pharyngeal swabs of primary pathogens associated with URTD in cats by microbiologic culture or nucleic acid amplification

| | Microbiologic culture (*n* Positive/*n* total assayed, %) | | | PCR assay (*n* Positive/*n* total assayed, %) | | |
|---|---|---|---|---|---|---|
| | Nasal | Pharyngeal | Kappa | Nasal | Pharyngeal | Kappa |
| *Bordetella bronchiseptica* | 3/59, 5.1 | 0/59, 0 | 0 | n/a | n/a | n/a |
| FHV-1 | 37/54, 68.5 | 27/54, 50.0 | 0.63 | 49/60, 81.7 | 44/60, 73.3 | 0.57 |
| *Mycoplasma* species | 28/59, 47.5 | 31/59, 52.5 | 0.62 | 27/50, 54 | 33/51, 64.7 | 0.53 |
| Total* | 39/51, 76.5 | 39/51, 76.5 | 32/51, 62.7 | 41/45, 91.1 | 42/45, 93.3 | 29/45, 64.4 |

Samples were collected as in Table 1.
*Total represents results from cats in which all assays were performed.

both sampling sites were taken into account, there was a 64.3% (27 of 42) agreement rate for detection of *Mycoplasma* species between PCR assay and bacteriologic culture.

### *Detection of organisms in which no traditional primary pathogens were detected*

We considered FHV-1, FCV, *C felis*, *B bronchiseptica* primary pathogens because Koch's postulates have been fulfilled. When results of all assays and both sample sites were considered, at least one primary pathogen was cultured or amplified from all but nine cats (Table 3). In these nine cats, *Mycoplasma* species was detected in seven. Six of the *Mycoplasma* species positive cats were co-infected with *Pasteurella* species (*P multocida* [$n = 5$], *P dogmatis* [$n = 3$]) and four were co-infected with *Moraxella* species.

## Discussion

A primary goal of this study was to determine whether nasal or pharyngeal samples were

**Table 3**. Organisms isolated from nine cats with clinical signs of URTD in which no primary pathogens were detected

| Case | *Mycoplasma* species | | | | Aerobic culture | |
|---|---|---|---|---|---|---|
| | PCR assay | | Culture | | | |
| | Nasal | Pharyngeal | Nasal | Pharyngeal | Nasal | Pharyngeal |
| 1 | (+) | (+) | (+) | (+) | *Pasteurella multocida* | *Pasteurella multocida*, Non-hemolytic *Streptococcus*, *Flavobacterium*, *Haemophilus* |
| 2 | n/a | n/a | (−) | (−) | (−) | *Pasteurella dogmatis* |
| 3 | n/a | n/a | (+) | (+) | (−) | (−) |
| 4 | n/a | n/a | (−) | (+) | (−) | *Pasteurella multocida*, *Moraxella*, *Simonsiella* |
| 5 | (−) | (+) | (−) | (−) | (−) | *Pasteurella multocida*, *Flavobacterium* |
| 6 | n/a | n/a | (−) | (−) | (−) | *Moraxella*, *Simonsiella*, *Bacillus* |
| 7 | (+) | (−) | (−) | (−) | Non-hemolytic *Streptococcus* | *Pasteurella multocida*, *Moraxella*, *Pasteurella dogmatis* |
| 8 | n/a | n/a | (−) | (+) | (−) | *Pasteurella multocida*, *Moraxella*, *Pasteurella dogmatis* |
| 9 | (−) | (−) | (−) | (+) | (−) | *Moraxella*, *Pasteurella dogmatis* |

Samples were collected as in Table 1. Primary pathogens were defined as: *Bordetella bronchiseptica*, *Chlamydophila felis*, FCV, FHV-1.

optimal for detection of the primary pathogens associated with URTD in shelter cats; detection rates of pathogenic organisms obtained by pharyngeal and nasal swabs were similar between sites for all traditionally defined primary pathogens. With the exception of *Bordetella bronchiseptica*, the kappa statistics for comparison of nasal and pharyngeal sampling sites for all assays and organisms were classified as moderate (FHV-1 PCR, *Mycoplasma* species PCR), substantial (FHV-1 virus isolation, *Mycoplasma* species culture, FCV), or perfect (*C felis*). In such a poorly distributed population (FCV, *C felis*, *B bronchiseptica*), the kappa statistic must be viewed with caution. Nasal swabs performed in the manner used for this study require a great deal more time and discomfort for the cat with minimal improvement in yield. Therefore, unless there is a high index of suspicion of FHV-1 and virus isolation is more readily available, swab sampling sites are most likely equivalent. However, to achieve maximal sensitivity for use in prevalence studies, results from both sample sites are needed. It must be noted that swabs of different types were used for sampling from the two anatomic sites and that inhibition of both nucleic acid amplification and microbiologic culture has been reported depending on swab type. However, as results are similar between the two anatomic sites, it is doubtful that results would have differed. Additionally, given the small size of the nares of a cat, we feel that the urethral culture swab is ideally suited to sampling at that site.

As FHV-1, FCV, and *Mycoplasma* species require specialized sample handling to maximize yield and require minimal transport time before recovery rates fall, nucleic acid amplification assays are being used more frequently. In a previous study, *Mycoplasma* species culture and PCR assay results were compared in a small number of samples taken from the nasal cavity of cats of unknown respiratory health status and shown to be similar (Johnson et al 2004). In this study, we compared results of culture and PCR assay for FHV-1 and *Mycoplasma* species in a larger number of cats with URTD and determined the techniques to give similar results although the kappa statistics for *Mycoplasma* species were not as high as previously reported (Johnson et al 2004). While PCR assays present several distinct advantages over culture (decreased need for special transport, time for storage, rapid results), the assays detect only the presence of microbial nucleic acid, not viable organisms. However, it is unlikely that DNA of dead organisms persist for any length of time. We conclude that PCR assays for both FHV-1 and *Mycoplasma* species are acceptable alternatives to traditional culture techniques for use in prevalence studies.

Because FHV-1 was so prevalent in the cats of this study, most cats with concurrent infections were FHV-1 positive. FHV-1 can be a primary pathogen and can also damage epithelial tissues allowing for secondary bacterial infection. In addition, currently available assays cannot discriminate between FHV-1 modified live vaccine strains and virulent strains (Maggs and Clarke 2005). Thus, detection of FHV-1 by culture or PCR assay does not prove illness associated with the virus. In addition, while FHV-1 could be grown or amplified from similar numbers of nasal and pharyngeal samples, maximum sensitivity was not achieved until results of both sites were combined. Because FHV-1 culture and conventional PCR assays are expensive and positive results do not correlate to illness, routine use of these assays in individual shelter cats with URTD appears to have little clinical benefit. Results of quantitative PCR assays that document the FHV-1 viral load in a sample may prove to have a better positive predictive value (Veir et al 2003), but further studies are needed.

The prevalence of FCV in this study population was lower than in previous studies. Even in normal cats, the rate of detection of FCV in the oropharynx via RT-PCR or virus isolation has been reported to be as high as 22% (Binns et al 2000) as the virus can be shed continuously despite lack of clinical signs. It is possible to have false-negative RT-PCR assay results because of RNA degradation. However, the samples were handled appropriately and the two RT-PCR assays gave similar results. In addition, while our virus isolation assay does not discriminate between FCV and FHV-1, all samples positive on virus isolation were also positive for FHV-1 via PCR assay. Based on these findings, we believe that FCV was not a common cause of disease in this population of cats. As for FHV-1, positive FCV culture or RT-PCR assay results do not correlate to FCV-associated disease and the assays are relatively expensive. Based on these facts, routine use of FCV culture or RT-PCR assay in individual shelter cats with acute URTD appears to have minimal clinical benefit.

Both the pharynx and nasal cavity of normal and clinically affected cats are host to a wide

variety of organisms (Bannasch and Foley 2005, Johnson et al 2005). Thus, detection of different aerobic bacteria from the nasal cavity and pharynx of the cats described here was not unexpected. Results of this study are similar to others and suggest that aerobic culture of nasal discharge or the pharynx of cats with URTD is unlikely to yield useful clinical information and that it may be more cost effective to use logical empirical antibiotic therapy when bacterial URTD is suspected. In a previous study of antibiotic susceptibility testing of aerobic bacterial isolates from cats with URTD, more than 80% of the aerobic bacterial isolates from both the upper and lower airway cultures were susceptible to amoxycillin–clavulanic acid, cephalosporins, chloramphenicol, enrofloxacin, and tetracyclines (Stein and Lappin 2001).

All of the organisms isolated from the cats described here have also been isolated from healthy cats; therefore, determining which are primary pathogens should be based on demonstration of Koch's postulates. While these have been fulfilled for FHV-1, FCV, *B bronchiseptica*, and *C felis*, other proposed primary pathogens (*Mycoplasma* and *Pasteurella* species) have only been implicated by epidemiologic evaluations (Bannasch and Foley 2005, Johnson et al 2005). In this study, only nine cats were negative for all the primary pathogens; in seven of these cats *Mycoplasma* species were detected either by PCR assay or bacteriologic culture. In the remaining two cats, *Pasteurella* and *Moraxella* species were the only organisms associated with URTD detected (Table 3). *Pasteurella multocida* has been suggested to be associated with chronic nasal disease in cats by one author (Johnson et al 2005) and is associated with atrophic rhinitis in swine (Eamens et al 1988). Similarly, *Moraxella* species are associated with rhinitis in humans (Boyle et al 1991), but no evidence for their role as a primary pathogen in acute upper respiratory disease in cats has been published. It is possible that an unknown organism or separate disease process (allergic, foreign body, nasopharyngeal polyp, or anatomic abnormality) may have caused the clinical signs in these nine cats. For example, some authors have suggested that anaerobic bacteria may be associated with disease in cats with chronic rhinitis (Johnson et al 2005) and we did not culture for that group of bacteria. However, it is also possible that some *Mycoplasma*, *Pasteurella* or *Moraxella* species can be primary pathogens in cats. Further studies attempting experimental transmission and proof of Koch's postulates with these organisms will be needed to prove or deny the hypothesis.

We failed to detect *C felis* in any sample. *Chlamydophila felis* is most often associated with conjunctivitis and ocular signs in cats (Bannasch and Foley 2005) and while this was not an exclusionary criterion in our study, very few cats exhibited conjunctivitis. The results of this suggest that *C felis* was not a common cause of URTD in these cats and that performance of assays to detect *C felis* on nasal or pharyngeal swabs is unlikely to have significant clinical benefit.

The data reported in this study suggest that either the nasopharynx or rostral nasal cavity are suitable sampling sites for cats with URTD in the shelter situation if the causative agent of any current outbreaks is not known. Additionally, we demonstrated that nucleic acid amplification techniques are at least equivalent in detection of primary pathogens when compared to microbiologic culture and can be used if it is more convenient and economical. The presence of mycoplasmal species in seven cats with no evidence of primary pathogens suggests these organisms may play a significant causative role in URTD in the shelter setting.

## References


Baird SC, Carman J, Dinsmore RP, Walker RL, Collins JK (1999) Detection and identification of *Mycoplasma* from bovine mastitis infections using a nested polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation* **11**, 432–435.

Bannasch MJ, Foley JE (2005) Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. *Journal of Feline Medicine and Surgery* **7**, 109–119.

Binns SH, Dawson S, Speakman AJ, Cuevas LE, Hart CA, Gaskell CJ, Morgan KL, Gaskell RM (2000) A study of feline upper respiratory tract disease with reference to prevalence and risk factors for infection with feline calicivirus and feline herpesvirus. *Journal of Feline Medicine and Surgery* **2**, 123–133.

Boyle FM, Georghiou PR, Tilse MH, McCormack JG (1991) *Branhamella (Moraxella) catarrhalis*: pathogenic significance in respiratory infections. *Medical Journal of Australia* **154**, 592–596.

Burgesser KM, Hotaling S, Schiebel A, Ashbaugh SE, Roberts SM, Collins JK (1999) Comparison of PCR, virus isolation, and indirect fluorescent antibody staining in the detection of naturally occurring feline herpesvirus infections. *Journal of Veterinary Diagnostic Investigation* **11**, 122–126.

Cape L (1992) Feline idiopathic chronic rhinosinusitis: a retrospective study of 30 cases. *Journal of the American Animal Hospital Association* **28**, 149–155.

Eamens GJ, Kirkland PD, Turner MJ, Gardner IA, White MP, Hornitzky CL (1988) Identification of toxigenic *Pasteurella multocida* in atrophic rhinitis of pigs by in vitro characterization. *Australian Veterinary Journal* **65**, 120–123.

Feinstein AR (2002) Principles of Medical Statistics. Boca Raton, FL: CRC Press LLC.

Foley J, Bannasch MJ (2004) Infectious diseases of dogs and cats. In: Miller LS, Zawitowski S (eds), *Shelter Medicine for Veterinarians and Staff*. Ames: Blackwell Publishing, pp. 235–284.

Foster SF, Barrs VR, Martin P, Malik R (1998) Pneumonia associated with *Mycoplasma* spp in three cats. *Australian Veterinary Journal* **76**, 460–464.

Foster SF, Martin P, Braddock JA, Malik R (2004) A retrospective analysis of feline bronchoalveolar lavage cytology and microbiology (1995–2000). *Journal of Feline Medicine and Surgery* **6**, 189–198.

Helps CR, Lait P, Damhuis A, Bjornehammar U, Bolta D, Brovida C, Chabanne L, Egberink H, Ferrand G, Fontbonne A, Pennisi MG, Gruffydd-Jones T, Gunn-Moore D, Hartmann K, Lutz H, Malandain E, Mostl K, Stengel C, Harbour DA, Graat EA (2005) Factors associated with upper respiratory tract disease caused by feline herpesvirus, feline calicivirus, *Chlamydophila felis* and *Bordetella bronchiseptica* in cats: experience from 218 European catteries. *Veterinary Record* **156**, 669–673.

Johnson LR, Drazenovich NL, Foley JE (2004) A comparison of routine culture with polymerase chain reaction technology for the detection of *Mycoplasma* species in feline nasal samples. *Journal of Veterinary Diagnostic Investigation* **16**, 347–351.

Johnson LR, Foley JE, De Cock HE, Clarke HE, Maggs DJ (2005) Assessment of infectious organisms associated with chronic rhinosinusitis in cats. *Journal of the American Veterinary Medical Association* **227**, 579–585.

Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. *Biometrics* **33**, 159–174.

Maggs DJ, Clarke HE (2005) Relative sensitivity of polymerase chain reaction assays used for detection of feline herpesvirus type 1 DNA in clinical samples and commercial vaccines. *American Journal of Veterinary Research* **66**, 1550–1555.

Padrid PA, Feldman BF, Funk K, Samitz EM, Reil D, Cross CE (1991) Cytologic, microbiologic, and biochemical analysis of bronchoalveolar lavage fluid obtained from 24 healthy cats. *American Journal of Veterinary Research* **52**, 1300–1307.

Pedersen NC, Sato R, Foley JE, Poland AM (2004) Common virus infections in cats, before and after being placed in shelters, with emphasis on feline enteric coronavirus. *Journal of Feline Medicine and Surgery* **6**, 83–88.

Radford AD, Bennett M, McArdle F, Dawson S, Turner PC, Glenn MA, Gaskell RM (1997) The use of sequence analysis of feline calicivirus hypervariable region in the epidemiological investigation of FCV related disease and vaccine failures. *Vaccine* **12/13**, 1451–1458.

Ruch-Gallie R, Veir JK, Spindel M, Lappin MR (2008) Efficacy of amoxycillin and azithromycin for the empirical treatment of cats with suspected bacterial upper respiratory infections. *Journal of Feline Medicine and Surgery* **10**, 542–550.

Stein JE, Lappin MR (May 2001) Bacterial culture results in cats with upper and lower airway disease: 255 cases (1995–1999). *American College of Veterinary Internal Medicine Annual Forum* (abstract).

Sykes JE, Studdert VP, Anderson G, Browning GF (1997) Comparison of *Chlamydia psittaci* from cats with upper respiratory tract disease by polymerase chain reaction analysis of the ompA gene. *Veterinary Record* **140**, 310–313.

Sykes JE, Studdert VP, Browning GF (1998) Detection and strain differentiation of feline calicivirus in conjunctival swabs by RT-PCR of the hypervariable region of the capsid protein gene. *Archives of Virology* **143**, 1321–1334.

Veir JK, Dow SW, Lappin SW (2003) Detection of feline herpesvirus-1 DNA from swabs collected from the pharynx or nasal discharges of cats using fluorogenic and conventional PCR. *Journal of Veterinary Internal Medicine* **17**, 425 (abstract).