Journal of Feline Medicine and Surgery (2004) **6**, 189–198

jfms

www.elsevier.com/locate/jfms

A retrospective analysis of feline bronchoalveolar lavage cytology and microbiology (1995–2000)

S.F. Foster[a,*], P. Martin[b], J.A. Braddock[b], R. Malik[b]

[a] *Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6012, Australia*
[b] *University Veterinary Centre Sydney, The University of Sydney, NSW 2006, Australia*

Summary A retrospective study of 88 bronchoalveolar lavage specimens from 80 cats presenting to the University of Sydney Veterinary Centre between 1995 and 2000 was performed. Bronchoalveolar lavage cytology and microbiology in conjunction with other diagnostic aids and patient records were used to classify cases as lower respiratory tract infections (LTRIs), LRTI revisits, feline bronchial disease, cardiac disease, neoplastic disease and multisystemic disease. Cases for which a definitive diagnosis could not be made were classified as inconclusive. Infectious agents identified were *Mycoplasma* spp., *Pasteurella* spp., *Bordetella bronchiseptica*, *Salmonella typhimurium*, *Pseudomonas* sp., *Cryptococcus neoformans*, *Aelurostrongylus abstrusus* and *Eucoleus aerophilus*. The study highlights the importance of bronchoalveolar lavage cytology and microbiology in the evaluation of feline lower respiratory tract disease.

Introduction

Feline lower respiratory tract (LRT) disease can have diverse causes including infection, cardiac disease, neoplasia and trauma. Many cases, however, have inflammatory airway disease with no identifiable aetiology which is variably termed feline asthma, feline asthma syndrome, feline bronchial disease (FBD) or chronic bronchitis. FBD has a wide range of clinical, clinicopathologic and radiographic features making definitive diagnosis, therapy and prognosis difficult (Corcoran et al., 1995; Dye et al., 1996; Moise et al., 1989).

Three retrospective studies and one prospective study have attempted to define FBD further (Corcoran et al., 1995; Dye et al., 1996; Moise et al., 1989; Simard and Dube, 1992) but routine bacterial culture was only performed in one study (Dye et al., 1996) and routine mycoplasmal cultures were not performed. The role of mycoplasmas is being increasingly examined in human asthma where there are strong associations between (i) mycoplasmal infection and exacerbation of asthma (ii) chronic mycoplasmal infections and asthma and (iii) induction of asthma subsequent to mycoplasmal infection (Gil et al., 1993; Kondo et al., 1994; Kraft et al., 1998; Micillo et al., 2000; Petrovsky, 1990; Sabato et al., 1984; Seggev et al., 1986, 1996; Teo et al., 1986; Yano et al., 1994). In order to define potential associations between mycoplasmal infections and FBD in cats, associations between mycoplasmal infections and other types of bronchopulmonary diseases must also be examined.

Common isolates in cats with bacterial pneumonia are reported to include *Pasteurella multocida*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bordetella bronchiseptica* and *Streptococcus canis* (Henik and Yeager, 1994). However, bacterial pathogens in feline pneumonia have been poorly documented (Greene, 1998) and an extensive literature search revealed only one detailed retrospective study of infectious causes of pneumonia in cats (Bart et al., 2000). The majority of cases in the study were kittens less than 12 weeks old and the bacteria

* Corresponding author. Current address: PO Box 598, Devonport, Tasmania 7310, Australia
E-mail address: sffoster@southcom.com.au (S.F. Foster).

1098-612X/04/$ - see front matter © 2004 Published by Elsevier Ltd on behalf of ESFM and AAFP.
doi:10.1016/j.jfms.2003.12.001

identified were *B. bronchiseptica*, *Pasteurella* spp., *Mycoplasma* spp., *E. coli* and *Streptococcus* spp.

Bronchoalveolar lavages (BALs) were performed on cats with a variety of LRT diseases at the University of Sydney Veterinary Centre (UVCS). This retrospective study was performed in an attempt to define the cytological and microbiological features of LRT diseases in cats.

Materials and methods

Records of BAL cytology and microbiology for specimens collected from cats between 1995 and 2000 at UVCS were compiled by one of the authors (PM). For the BAL specimens submitted, patient records were reviewed. Each BAL was regarded as an individual case. Records for each case were considered complete if a definitive diagnosis had been made by a combination of history, clinical signs, diagnostic investigations and/or response to treatment. Attempts were made to follow-up cases for which a definitive diagnosis could not be determined from the patient records.

All BALs, except one, were performed in a standard manner. The cats were anaesthetised and a sterile endotracheal tube passed. A sterile dog urinary catheter (6—8 French gauge) was passed gently through the endotracheal tube until it wedged in a distal airway and usually two, 5—10 ml aliquots of sterile saline were then flushed through the catheter. Fluid was retrieved from the catheter by syringe aspiration. Fluid that was coughed through the sterile endotracheal tube was also collected into a sterile container to ensure adequate sample volume for assessment although it was not typically processed. One cat was considered too dyspnoeic for anaesthesia and had a transtracheal aspirate performed under local anaesthesia.

For each sample, two drops of lavage fluid containing mucus were placed on a microscope slide and unstained wet preparations were examined microscopically for the presence of parasites. Multiple squash preparations of mucus from each lavage specimen were air dried and stained with Diff Quik. The BAL cytology preparations were analysed for the presence of mucus, oropharyngeal squames, oropharyngeal bacteria, respiratory pathogens, blue pigment in macrophages, epithelial cells and inflammatory cells. Total cell counts were not performed as reference ranges had not been validated for this method of sample collection. Inflammatory cell numbers were subjectively assessed as many, moderate, few or none. Differential cell counts for BAL cytology were classified by the predominant inflammatory cell type, if greater than or equal to 50% of the total, or described as mixed, if no cell type predominated. Epithelial cells were not considered in the calculation of relative cell counts following the recommendations of the American Thoracic Society for reporting cell counts in humans (American Thoracic Society, 1990). If sufficient material was submitted, several smears were examined from each sample.

Prior to June 1998, only BALs with neutrophilic inflammation or with micro-organisms evident on cytological examination were cultured. After June 1998, all samples were cultured regardless of BAL cytology. If bacteria were seen in Diff Quik stained smears, a Gram stain (Burke's modification) was performed and the lavage was cultured both aerobically and anaerobically on sheep blood agar at 37 °C. If no bacteria were seen, only aerobic incubation was undertaken. When fungi were identified in smears, lavage fluid was additionally cultured on Sabouraud dextrose agar at 37 °C and 28 °C. Plates were incubated for at least seven days prior to reporting negative growth. Identification of mycoplasmas was based on colonial morphology and the inability to see bacterial forms with Gram stain. Other bacteria were identified using standard microbiological techniques.

Pure culture of any bacterium or fungus was considered significant as was moderate to heavy growth of any microbe with minimal growth of oral contaminants. However, the infectious agent in cases with a positive microbial culture was only considered the aetiological agent of the disease if historical, clinical, radiographic and cytologic findings were supportive and if there was an unambiguous response to appropriate antimicrobial treatment. These cases and those in which parasites were identified cytologically were termed lower respiratory tract infections (LRTIs).

The remaining cases were classified as FBD, cardiac disease, neoplastic disease, LRTI revisits, multisystemic disease or inconclusive. The criteria for FBD included lack of identification of an infectious cause and either histopathology or ongoing clinical signs requiring constant or intermittent bronchodilator, antiinflammatory and/or immunosuppressive therapy. Neoplastic cases were diagnosed by BAL cytology, fine-needle aspirate cytology and/or histopathology. Cardiac cases were identified by radiography and echocardiography. Multisystemic disease cases were identified with clinical pathology and histopathology. BALs performed specifically to check treatment efficacy for previously identified infectious agents were

Table 1 Bronchoalveolar lavage cytology results

Case type	No.	Mucus	Squames	OPB	Inflammatory cells					N	H	E	L	Mixed	Macrophage pigment
					Many	Mod	Few	None	NR						
LRTI	19	19	6	0	18	1				17	2				3
LRTI revisit	2	2	1	0	2						1			1	2
FBD	26	26	10	4	23	2			1	13	3	8		2	11
Cardiac	2	2	1	0	1	1					2				2
Neoplastic	8	8	2	1	2	2	3	1		2	2		1	2	4
Multisystemic	3	3	1	1		3				1	1			1	2
Inconclusive	28	26	8	5	19	6	3			12	6	3		7	11
Total	88	86	29	11	65	15	6	1	1	45	17	11	1	13	35

Abbreviations: LRTI=lower respiratory tract infection; FBD=feline bronchial disease; OPB=oropharyngeal bacteria; Mod=moderate; NR=not recorded; N=neutrophilic; H=histiocytic; E=eosinophilic; L=lymphocytic.

classified as LRTI revisits. Cases were regarded as inconclusive for one of the following reasons: signs resolved without treatment and did not recur; signs resolved after a single course of empirical single-agent or multi-agent therapy; signs resolved with antibiotics but recurred occasionally and resolved either spontaneously or after a short-course of antibiotic treatment; lost to follow-up because owners could not be contacted or because death had occurred due to unrelated or unknown causes.

BAL cytology and microbiology were analysed for each disease category. Information about prior antibiotic therapy and serological testing with commercial ELISA and immunomigration kits for feline leukaemia virus (FeLV) antigen, feline immunodeficiency virus (FIV) antibody and heartworm antigen was also included if known, as these factors could have potentially affected cytology and microbiology results for the disease categories.

Results

Eighty-eight unguided BAL specimens were collected from 80 cats between 1995 and 2000; eight cats had two BALs. Radiographic and/or histopathologic evidence of LRT disease were present in all cases except two, both of which had compelling clinical evidence of LRT disease. Radiographic details for the LRTI and FBD cases are published elsewhere (Foster et al., 2004a,b).

Sixty-seven of 88 specimens were cultured for bacteria and fungi. One author (PM) performed cytology on 62 of the 88 (70%) samples and microbiology on 66 of the 67 cultured specimens (99%). Cytology on the other 16 samples was performed by a second pathologist. The number of cases per year for the years 1995 to 2000 were 1, 11, 13, 28, 22 and 13 respectively.

The 88 cases were classified as follows: FBD (26), LRTI (19), neoplastic disease (8), multisystemic disease (3), LRTI revisits (2), cardiac disease (2) and inconclusive (28).

BAL cytology

The cytology is detailed in Table 1. Oropharyngeal bacteria were noted in 11 cases, all of which also had oropharyngeal squames. Macrophages were noted to contain blue pigment in 35 cases and neoplastic epithelial cells were detected in two samples.

BAL microbiology and parasitology

Microbiological results from the 67 cases cultured were as follows: no growth (16), mixed contaminants (28), *Mycoplasma* spp. (15), *Pasteurella* spp. (5), *Salmonella typhimurium* (2, both from cats with concurrent *Aelurostrongylus abstrusus* infection), *Cryptococcus neoformans* var. *grubii* (2), *Pseudomonas* sp. (1) and *B. bronchiseptica* (1). Parasitic agents identified in the lavage specimens were *A. abstrusus* (2) and *Eucoleus aerophilus* (previously *Capillaria aerophila*) (1).

Thirteen of the 15 cases from which mycoplasmas were cultured were classified as LRTIs and two were considered inconclusive. One of the inconclusive cases had only light to moderate growth of mycoplasmas and cytological evidence of oropharyngeal contamination. The predominantly eosinophilic inflammation in this case was more suggestive of FBD. Successful therapy on this

occasion and for a recurrence one year later, consisted of both doxycycline and corticosteroids. The second cat which had only a moderate growth of mycoplasmas and the possibility of sample contamination (10 mixed colonies), was lost to follow-up. Two cases of mycoplasmal LRTI had mixed infections. One had a heavy growth of mycoplasmas and a moderate growth of *Pasteurella multocida* and one had heavy growth of both mycoplasmas and *B. bronchiseptica*.

Pasteurella spp. were cultured from five BALs but two specimens with light pure growths, had cytological evidence for oropharyngeal contamination. One of these cases, with a clinical history consistent with FBD, was lost to follow up and classified as inconclusive. The other was classified as multisystemic disease. The remaining three cases were classified as LRTIs. *Pasteurella* spp. in these cases were cultured with mixed anaerobes (1), a heavy growth of mycoplasmas (1) and with an unidentified Gram-negative bacterium (1).

S. typhimurium was cultured from two cases, one of which has been reported previously (Barrs et al., 1999). Both cases had concurrent *A. abstrusus* and the unreported case also had *Pseudomonas* sp. cultured. Both were classified as LRTIs.

Cryptococcus neoformans var. *grubii* (formerly var. *neoformans* serotype A) was cultured from two cats. One of these, reported previously (Barrs et al., 2000), was a FIV-infected cat that developed a LRTI due to *E. aerophilus* subsequent to successful treatment for pulmonary cryptococcosis; it was later euthanased with disseminated mast cell tumour. The cryptococcal and *E. aerophilus* infections were both classified as LRTIs. Only two colonies of *Cryptococcus neoformans* var. *grubii* were cultured from the second cat but it had a latex cryptococcal antigen agglutination test (LCAT) titre of eight. This cat's coughing had not responded to a four-week course of doxycycline and it had been treated with a single dose of subcutaneous ivermectin five days prior to its BAL. One month later the LCAT had increased to 16, so it was treated with itraconazole until the LCAT was zero. The cough resolved one day prior to the BAL and never recurred so was thought to be more likely due to a parasitic infection than due to cryptococcosis thus the case was considered inconclusive. The cat was serologically negative for FIV and FeLV but it had chronic renal failure, a sub-clinical urinary tract infection and was euthanased 12 months later because of pancreatic carcinoma.

Twenty-three of the 29 BAL samples with squames were cultured and 14 grew mixed contaminants. Of the 11 cases with oropharyngeal bacteria cytologically, eight were cultured and seven yielded mixed contaminants. Fifteen samples that grew contaminants had no squames or oropharyngeal bacteria noted cytologically.

Details for each disease category

LRTI

BAL cytology was neutrophilic in 17 cases and histiocytic in two. The two cases with histiocytic inflammation were both from the same cat (the FIV-positive cat with cryptococcosis followed by *E. aerophilus* infection). Eosinophils comprised less than 20% of the cell population in all samples except one, in which certain areas of the smear had up to 34% eosinophils. Infectious agents were observed cytologically except in the mycoplasmal LRTIs.

Four cats that had been diagnosed as LRTI and successfully treated (three with mycoplasmas and one with a mixed infection) had a recurrence of clinical signs and had second BALs performed 8–19 months (mean 14) after the initial BAL. Three of these subsuequent BALs were classified as inconclusive and one (from a mycoplasmal LRTI) was classified as FBD.

Serological testing for FIV antibodies was performed in nine cats (10 cases) and was positive in two cats (three cases). Serological testing for FeLV was negative in seven cases tested. Heartworm antigen testing was negative in three cases tested.

LRTI revisits

Two cats had BALs performed to check microbiological cure whilst being treated with antibiotics: one with *Mycobacterium thermoresistibile* (diagnosed from lung fine needle aspirate microbiology) (Foster et al., 1999) and one with *S. typhimurium*, *Pseudomonas* sp. and *A. abstrusus*. The BAL was histiocytic in the mycobacterial LRTI revisit and mixed (predominantly macrophages and neutrophils) in the case of salmonellosis. Culture was negative in both cases.

Feline bronchial disease

BAL cytology was neutrophilic (13), eosinophilic (8) (all with at least 60% eosinophils), histiocytic (3) and mixed (2). Fourteen of the 26 cases were cultured and five had no growth and nine had mixed contaminants. Of the 14, five had had no previous antibiotic therapy, six had been previously treated with doxycycline (one also receiving clindamycin),

one had received enrofloxacin and two had received unspecified antibiotics. Serological testing for FIV in five cats, heartworm antigen in six and FeLV in two proved negative. One cat (previously diagnosed with mycoplasmal LRTI) was FIV-positive.

Two of the cats with FBD had two BALs performed and had different cytology on each occasion. One cat had a mixed inflammatory response on the first occasion and a neutrophilic cytology on the second. On both occasions the cat was classified as having FBD. In the second cat, the initial BAL was predominantly eosinophilic and was classified as FBD. The second BAL, ten days before it died and five months after the first sample, was classified as LRTI. It was predominantly neutrophilic and *Pasteurella* sp. and mixed anaerobes were cultured. This cat had no signs of respiratory disease until three weeks after initiating potassium bromide therapy for intractable seizures.

Neoplastic disease

Eight cases of neoplastic disease were diagnosed: pulmonary carcinoma or adenocarcinona (5), nasal lymphosarcoma (1), laryngeal lymphosarcoma (1) and multicentric lymphosarcoma with pulmonary involvement (1). The differential cell count was neutrophilic in two cases (carcinoma, nasal lymphosarcoma), histiocytic in two (both carcinomas), mixed in two (carcinoma, laryngeal lymphosarcoma) and lymphocytic in the case with multicentric lymphosarcoma; one case (carcinoma) had no inflammatory cells. The neutrophilic differential cell count in the carcinoma case may have been influenced by the trans-tracheal mode of collection. Of the eight samples, three were not cultured and four had negative cultures (two from cats that had received prior antibiotics). One sample, from a cat that had had prior antibiotics, had a light growth of contaminants. Three cats (two with carcinomas and one with laryngeal lymphosarcoma) had serological testing for FIV and FeLV; all were negative.

Carcinoma was diagnosed cytologically from one TTA (confirmed histologically) and two BALs (one confirmed histologically). In the other two cases, the BALs were not diagnostic and diagnosis was made from lung fine-needle aspirate cytology or histology. Pulmonary lymphosarcoma was strongly suspected from BAL cytology in one case and this was confirmed histologically. Nasal lymphosarcoma was diagnosed by histopathology and laryngeal lymphosarcoma, by fine-needle aspirate cytology. The former case had evidence of LRT disease on thoracic radiographs but this may or may not have been related to the neoplastic process. The latter may not have had LRT involvement as there were scant white cells and equivocal thoracic radiographic evidence of LRT involvement.

Cardiac disease

Two cases of cardiac disease were diagnosed: one with excessive left ventricular moderator bands, the other with hypertrophic cardiomyopathy. The BALs were histiocytic in both cases. One sample was not cultured and the other grew oral contaminants; neither case had had prior antibiotics.

Multisystemic disease

Three cases were diagnosed with multisystemic disease. Feline infectious peritonitis (FIP) was diagnosed histologically in one cat. One cat had multiple eosinophilic granuloma complexes, eosinophilic rhinitis and eosinophilic and neutrophilic pulmonary inflammation; eosinophilic infiltration was not present in lymph nodes or liver thus hypereosinophilic syndrome was not diagnosed. The third cat had FIV infection, glomerulonephritis, unclassified cardiomyopathy and terminally, hepatic failure and neurological signs. The cat with FIP had a neutrophilic BAL and the FIV-infected cat had a histiocytic BAL. The BAL from the cat with eosinophilic skin and airway disease had a mixed inflammatory pattern (neutrophils, up to 50% eosinophils and macrophages) which corresponded well to its histopathological appearance. Culture yielded light growth of contaminants in two specimens and a light pure growth of *Pasteurella* sp. in the third. Two of the cases had no prior antibiotic therapy and one had had a variety of antibiotics at unspecified times.

Inconclusive cases

BAL cytology in the 28 inconclusive cases was classed as neutrophilic (12), histiocytic (6), eosinophilic (3) or mixed (7). Twenty-three samples were cultured and results were: no growth (5, two of which had had recent treatment with doxycycline or enrofloxacin), mixed contaminants (14), *Mycoplasma* spp. (2), *Pasteurella* sp. (1) and *C. neoformans* var. *grubii* (1). Serological testing for FIV (3), FeLV (2), and heartworm antigen (2) were negative in each case.

Discussion

This is the first study to analyse BAL cytology and microbiology from a variety of LRT diseases. Neutrophilic inflammation was the most common

finding and occurred in 50% of FBD cases. All LRTI cases except the FIV-infected cat with cryptococcosis followed by lungworm infection, displayed neutrophilic inflammation, suggesting that the normal response to pulmonary infection is neutrophilic unless there is concurrent immunosuppression. Only four of the LRTIs were diagnosed prior to June 1998 so a selection bias of neutrophilic samples for culture did not significantly affect results in the LRTI category.

None of the three cats with confirmed parasitic infections had an eosinophilic BAL and in all three cases, eosinophils comprised less than 21% of the cell count. It may be that parasitic infections elicit neutrophilic responses in cats, however, other factors may have influenced this: the presence of concurrent salmonellosis in two and FIV infection in one.

Eosinophilic inflammation occurred in 31% of FBD cases and FBD accounted for 73% of eosinophilic samples. The remaining eosinophilic cases were classified as inconclusive but could have been due to FBD. This would suggest that eosinophilic inflammation (eosinophils comprising 50% or greater) in BALs should be predictive of FBD. In healthy cats, eosinophils have been reported as comprising 16±14% of the inflammatory cells (Hawkins et al., 1994) and 25±21% of total nucleated cells (Padrid et al., 1991). However, the range of eosinophil percentages in the latter study was 2–83% suggesting that higher percentages should not be overinterpreted. Regardless, the present study would indicate that a predominance of eosinophils is suggestive of FBD.

Histiocytic cytology was present in 19% of BAL samples. It was recorded in both cases with cardiac disease and occurred in all disease categories. Lymphocytic inflammation was observed in only one sample from a cat which had pulmonary lymphosarcoma. Lymphocytic inflammation was not noted in other studies on bronchial disease (Corcoran et al., 1995; Dye et al., 1996; McCarthy and Quinn, 1986; Moise et al., 1989) and a lymphocytic BAL may be suggestive of lymphosarcoma in cats.

Blue pigment was observed in a proportion or all of the macrophages in 40% of the samples. Although it was relatively less common in the LRTI cases, it did not help in distinguishing disease groups. Dye and others (1996) reported darkly pigmented intracytoplasmic particles in a BAL from a cat exposed to passive smoking. However, the blue pigment in our cases was more likely to be due to haemoglobin degradation as it stained positive for iron with Perl's stain (PM, unpublished observations).

FIV infection has been shown to alter BAL cytology (Cadoré et al., 1997; Hawkins et al., 1996). BALs on four FIV-infected cats with wasting, stomatitis and cachexia were predominantly histiocytic but with increased numbers of neutrophils and lymphocytes relative to controls (Cadoré et al., 1997). These cats had interstitial pneumonitis with neutrophilic and lymphocytic alveolitis but did not have concurrent pulmonary bacterial or fungal infections. Another study demonstrated airway neutrophilia and increased macrophage counts, especially in cats infected for more than four years, but the BAL specimens were not cultured (Hawkins et al., 1996). The FIV-positive cat in the present study which had cryptococcosis and then lungworm had histiocytic BALs on both occasions. Two other FIV-positive cats, (one with multisystemic disease and one with FBD) also had histiocytic BALs. All three cats had disease features suggestive of acquired immunodeficiency syndrome (AIDS). It is possible that immune dysfunction caused by FIV decreased the expected neutrophilic responses and that FIV status may need to be taken into account when interpreting BAL cytology. Alternatively, pathology in these cats may have been histiocytic, although that would not be typical for cryptococcosis or lungworm (Henik and Yeager, 1994) or, in this study, FBD.

BAL cytology appeared to be specific but insensitive for detecting pulmonary neoplasia. Three cases of carcinoma (2/3 confirmed histologically) were diagnosed from BAL or transtracheal aspirate cytology and a diagnosis of pulmonary lymphosarcoma was suspected cytologically and confirmed histologically. BAL cytology in two cases of carcinoma and two cases of non-pulmonary lymphosarcoma was non-diagnostic, however, there may not have been LRT neoplasia in the latter two cases.

FBD cases were selected by rigid criteria: lack of identification of an infectious cause and either histopathology or ongoing clinical signs requiring constant or intermittent bronchodilator, anti-inflammatory and/or immunosuppressive therapy. Cultures were not performed in 46% of these cats so it is possible that aetiological infectious agents were not identified, however, the responses of these cats to treatment were suggestive of ongoing bronchial inflammation. No doubt these criteria led to exclusion of some cats that could have had mild or intermittent FBD but in a retrospective study where lungworm testing and culture were not performed routinely on all samples these criteria served to ensure that only cats with true idiopathic inflammatory airway disease were included for

analysis. BAL cytology in cats with FBD was usually very inflammatory, typically neutrophilic (50%) or eosinophilic (31%). Neutrophilic and eosinophilic inflammation were the most common findings in two other studies (Dye et al., 1996; Moise et al., 1989) although a very low incidence of eosinophilic cytology was reported in one study (Corcoran et al., 1995). Interestingly, one cat developed FBD three weeks after potassium bromide therapy was initiated and it is now known that potassium bromide can induce FBD that may be fatal (Boothe et al., 2002), as it was in this case.

The bacteriology results were different from those reported previously. The bacteria reported as occurring in the lower airways of healthy cats include *Pasteurella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *B. bronchiseptica* and *Micrococcus* spp. (Dye et al., 1996; Padrid et al., 1991). The bacteria reported in reviews of bacterial pneumonia in cats include *P. multocida*, *E. coli*, *K. pneumoniae*, *B. bronchiseptica*, *Streptococcus canis*, *Mycoplasma* spp., mycobacteria and Eugonic Fermenter-4 (Bart et al., 2000; Henik and Yeager, 1994). In the present study, infectious bacterial agents identified were mycoplasmas, *Pasteurella* spp., *S. typhimurium*, *Pseudomonas* sp. and *B. bronchiseptica*. In addition, one of the LRTI revisits had had *Mycobacterium thermoresistibile* cultured from lung fine-needle aspirates.

There is no "gold standard" that can be used to make a clinical diagnosis of LRTI in cats or dogs (Peeters et al., 2000). Historical, physical, haematological and radiographic findings known to be compatible with LRTIs are often non-specific or are inconsistently present (Hawkins, 2000). A classification of LRTI was only made in this study if historical, clinical, radiographic, cytologic and microbiologic findings were supportive and if there was an unambiguous response to antimicrobial treatment. These cases may have had underlying airway or systemic pathophysiology predisposing them to LRTIs. However, the presence of predisposing pathophysiology does not preclude a diagnosis of LRTI.

Mycoplasmas were identified in a recent study on predominantly young cats as the third most common bacterial cause of feline pneumonia (Bart et al., 2000). Mycoplasmas were the most common cause of LRTI in our study, in which all patients were older than 14 weeks. The number of mycoplasmal infections may have been underestimated in our study as specific media for mycoplasmas was not used. The ability to grow mycoplasmas on non-specific media has been noted previously (Foster et al., 1998; Gruffydd-Jones, 1991) but the sensitivity of this method is unknown.

Whilst it is known that mycoplasmas are not found in the LRTs of healthy cats (Padrid et al., 1991; Randolph et al., 1993) and that they are found in the LRTs of cats with pulmonary disease (Randolph et al., 1993), their significance is often disregarded. It is interesting that in this study, mycoplasmas were not cultured from any LRT disease other than LRTIs or possibly FBD, if the LRTIs were in fact secondary to FBD. This would suggest that pulmonary pathology alone is inadequate to permit secondary infection with these organisms and that any association between FBD and mycoplasmal infection is specific.

The role of mycoplasmas in human respiratory disease is constantly being re-evaluated. *Mycoplasma pneumoniae* has long been known to provoke exacerbation of asthma in humans with stable disease (Kondo et al., 1994; Seggev et al., 1986; Teo et al., 1986). *M. pneumoniae* is associated with significant IgE responses and it has been suggested that *M. pneumoniae*-specific IgE may provoke exacerbation of bronchial asthma (Seggev et al., 1996). Mycoplasmas have also been implicated as causing post-infection asthma in humans (Petrovsky, 1990; Yano et al., 1994) and it has been demonstrated that *M. pneumoniae* affects bronchomotor tone even in non-asthmatic children (Sabato et al., 1984). In these children, significant bronchodilator responsiveness was present for a month after infection and decreased expiratory flow rates, indicating impaired airway function, were demonstrated three years after the initial infection. Ten percent of patients in this study who first wheezed with *M. pneumoniae* infection, wheezed subsequently. In another study, the isolation rate of *M. pneumoniae* was significantly greater in patients with asthma than in controls and it was suggested that *M. pneumoniae* could be one of the stimuli that trigger acute asthma as most of the cases from which *M. pneumoniae* were isolated had no family history of atopy (Gil et al., 1993). Most studies of mycoplasmal infection in humans are performed on the basis of culture or serum antibody titres. In culture and serum antibody negative patients, Kraft and others (1998) showed by polymerase chain reaction (PCR) technology that *M. pneumoniae* is present in the airways in greater than 55% of the asthmatics studied, suggesting that asthmatic patients appear to be chronically infected or colonised with *M. pneumoniae*. They concluded that there was a need to investigate whether this was an epiphenomenon due to airway

inflammation but favoured the hypothesis that infection with *M. pneumoniae* was a pathogenetic mechanism in asthma.

Whilst the response of mycoplasmal LRTIs to antimicrobial therapy is compelling evidence of the pathogenicity of mycoplasmas, only two cases resolved completely. Others had mild recurrences of coughing which required further treatment or resolved spontaneously. This could suggest that mycoplasmal LRTI occurred in cats with FBD. However, it may well be that mycoplasmal LRTI caused serious pathology and dysfunction, resulting in ongoing inflammatory bronchial disease and airway hyperresponsiveness as it does in humans (Sabato et al., 1984). Other LRTIs caused ongoing signs after the infections cleared, as was evident with the LRTI revisits.

It is difficult to comment on the role of *Pasteurella* spp. in LRTIs as in this study, infections were mixed, one occurring as a terminal event in a cat with FBD. *Pasteurella multocida* is a common inhabitant of the oral and upper respiratory mucous membranes in cats with a carrier rate of over 30% (Biberstein and Holzworth, 1987) and has been reported as a cause of feline pneumonia (Bart et al., 2000; Henik and Yeager, 1994).

Salmonellosis occurred twice in association with *A. abstrusus* in FIV-negative cats and it has been postulated that migrating lungworm larvae may act as carriers for intestinal bacteria (Barrs et al., 1999). The previously reported kitten had displayed gastrointestinal signs prior to LRTI (Barrs et al., 1999) but the second cat was an apparently healthy adult and had no history or signs of enteric salmonellosis.

Tracheal washing has been regarded as being less sensitive for lungworm diagnosis than faecal analysis by the Baermann technique. Larval numbers are relatively small, especially during the later stages of disease and may not be detected in the small amount of dilute BAL fluid (Pedersen, 1988). However, egg output decreases dramatically after 2–3 months and larvae are only excreted intermittently so even faecal analysis is insensitive and multiple (usually three) faecal samples have been recommended for analysis (Foster, 1998; Pedersen, 1988). Pedersen (1988) suggested that it can be very different to differentiate lungworm infection from chronic allergic bronchiolitis (FBD).

The lungs are considered the primary site of infection for cryptococcosis in humans (Malik et al., 2001) but pulmonary infection would appear to be quite rare in feline cyptococcosis (Gerds-Grogan and Dayrell-Hart, 1997; Malik et al., 1992; Medleau et al., 1995). In this study, cryptococcal infection was uncommon and immune dysfunction was likely in both cases in which *C. neoformans* var. *grubii*. was cultured. In humans, there is a distinct association between immune status and *C. neoformans* variety with *C. neoformans* var. *gatti* tending to occur in healthy hosts and *C. neoformans* var. *grubii* (formerly var. *neoformans*) occurring in immunocompromised hosts (Mitchell et al., 1995; Speed and Dunt, 1995).

Criticisms of the BAL collection and processing techniques could be made. Bronchoscopic lavage is regarded as the optimal technique for retrieving fluid and performing total cell counts from specific airways (Foster, 1998; Padrid, 2000). At UVCS, a modified BAL was utilised for reasons of procedure time (very short), cost (significantly cheaper than bronchoscopic collection) and safety (airway occlusion with bronchoscopes is far greater than with a dog urinary catheter). Total cell counts were not performed as they have not been validated for this method of collection although extrapolation from a very similar technique using 20 ml of lavage fluid would probably have been acceptable (McCarthy and Quinn, 1986). As well as being dependent on method of collection (Hawkins et al., 1994; Padrid et al., 1991), total cell counts in humans and cats are not necessarily consistent: sequential BAL sampling often results in variable total cell counts despite stable differential cell counts (Ettensohn et al., 1988a,b; McCarthy and Quinn, 1986). Cytological slides were not available for re-evaluation but 70% of the cytology and 99% of the microbiology were performed by a single pathologist thus ensuring a high level of consistency of procedure and reporting.

In the majority of our samples, a squash preparation of the mucus was prepared as recommended by Moise and Blue (1983) and Greene (1998). This technique has been criticised as these cells may or may not reflect the general nature of airway inflammation (Padrid, 2000). However, the inflammatory cytology of cytocentrifuged and direct-smear preparations of BAL fluid collected by bronchoscope may not correlate with histological findings either (Norris et al., 2002) and when histopathology was available in our cases, inflammatory cytology was consistent with that reported from histopathology.

Quantitative cultures were not performed. Airway specimens from cats are not consistently sterile. Quantitative growth of less than 10^4 colony forming units (CFU)/ml is regarded as commensal (Padrid et al., 1991) although, Dye and others (1996) cultured greater than 10^4 CFU/ml in a

number of healthy cats. As a standard inoculum (0.1 ml) was used for culture in each case in our study, it was estimated that a moderate to heavy growth of mycoplasmas was likely to represent at least 10^4 CFU/ml. In our study, apart from mycoplasmas, which are not normal inhabitants of the LRT, the aetiological agents of the LRTIs were observed cytologically; mycoplasmas do not have a cell wall so do not take up Gram stain. The importance of cytologic identification of intracellular bacteria in BAL cytology was recently emphasised in a study on canine BALs (Peeters et al., 2000) and we consider the Gram-staining of BAL smears (and other stains as appropriate) as critical in the diagnosis of bacterial LRTIs. It is surprising that such little emphasis has been placed upon this in previous feline studies and reviews (Dye et al., 1996; Moise and Blue, 1983; Padrid et al., 1991). The presence of oropharyngeal squames cytologically should alert suspicion of contamination but should not preclude culture as 39% of the samples with squames did not have mixed contaminants cultured. Samples with oropharyngeal bacteria noted cytologically were likely to have mixed contaminants cultured.

Whilst this study has much information that may be pertinent only to cats in Sydney, it is the only retrospective study to review BAL cytology and microbiology for a wide range of LRT diseases. In conclusion, the typically cited bacteria in feline pneumonia were not identified commonly and the majority of cats with LRTIs were infected with mycoplasmas. Cryptococcal LRTIs were recognised only in cats with suspected immune dysfunction and salmonellosis was identified with concurrent lungworm in two cases. BAL features of FBD were not significantly different from those reported elsewhere but emphasised that eosinophilic inflammation is more likely in FBD than in other diseases. The relationship of mycoplasmas to FBD could not be established conclusively in this retrospective study but mycoplasmas were not cultured from cats with neoplastic, cardiac or multisystemic diseases suggesting that pulmonary pathology per se is inadequate for mycoplasma colonisation. As mycoplasmal infection was not uncommon in this series of cats, it should not be overlooked as a cause of LRTI and a potentially inciting agent of FBD. Human rhinovirus, *Chlamydia pneumoniae* and *M. pneumoniae* are all known to exacerbate and perhaps cause asthma in humans (Micillo et al., 2000). The cat may well prove a useful model for infection-induced asthma although further prospective clinical studies, preferably using PCR technology, are necessary to determine the role of feline infectious agents and asthma.

Acknowledgements

This study would not have been possible without the enthusiastic assistance of Rhian Foster and Karen Kilpatrick in medical records. The clinicians who managed the cases are also gratefully acknowledged. Richard Malik is supported by the Valentine Charlton Bequest of the Post Graduate Foundation in Veterinary Science of the University of Sydney.

References

American Thoracic Society, 1990. Clinical role of bronchoalveolar lavage in adults with pulmonary disease. Am Rev Respir Dis 142, 481–486.

Barrs, V.R., Martin, P., Nicoll, R.G., Beatty, J.A., Malik, R., 2000. Pulmonary cryptococcosis and *Capillaria aerophila* infection in an FIV-positive cat. Aust Vet J 78, 154–158.

Barrs, V.R., Swinney, G.R., Martin, P., Nicoll, R.G., 1999. Concurrent *Aelurostrongylus abstrusus* infection and salmonellosis in a kitten. Aust Vet J 77, 229–232.

Bart, M., Guscetti, F., Zurbriggen, A., Pospischil, A., Schiller, I., 2000. Feline infectious pneumonia: a short literature review and a retrospective immunohistological study on the involvement of *Chlamydia* spp. and distemper virus. The Vet J 159, 220–230.

Biberstein, E.L., Holzworth, J., 1987. Bacterial diseases, in: Holzworth, J (Ed.), Diseases of the Cat. Philadelphia. Saunders, pp. 279–319.

Boothe, D.M., George, K.L., Couch, P., 2002. Disposition and clinical use of bromide in cats. J Am Vet Med Assoc 221, 1131–1135.

Cadoré, J.L., Steiner-Laurent, S., Greenland, T., Mornex, J.F., Loire, R., 1997. Interstitial lung disease in feline immunodeficiency virus (FIV) infected cats. Res Vet Sci 62, 287–288.

Corcoran, B.M., Foster, D.J., Luis Fuentes, V., 1995. Feline asthma syndrome: a retrospective study of the clinical presentation in 29 cats. J Small Anim Pract 36, 481–488.

Dye, J.A., McKiernan, B.C., Rozanski, E.A., Hoffmann, W.E., Losonsky, J.M., Homco, L.D., Wesiger, R.M., Kakoma, I., 1996. Bronchopulmonary disease in the cat: historical, physical, radiographic, clinicopathologic, and pulmonary functional evaluation of 24 affected and 15 healthy cats. J Vet Intern Med 10, 385–400.

Ettensohn, D.B., Jankowski, M.J., Duncan, P.G., Lalor, P.A., 1988a. Bronchoalveolar lavage in the normal volunteer subject. I. Technical aspects and intersubject variability. Chest 94, 275–280.

Ettensohn, D.B., Jankowski, M.J., Redondo, A.A., Duncan, P.G., 1988b. Bronchoalveolar lavage in the normal volunteer subject. II. Safety and results of repeated BAL, and use in the assessment of intrasubject variability. Chest 94, 281–285.

Foster, D., 1998. Diagnosis and management of chronic coughing in cats. In Pract 20, 261–267.

Foster, S.F., Martin, P., Allan, G.S., Barrs, V.R., Malik, R., 2004a. Lower respiratory tract infections in cats: 21 cases (1995–2000). JFMS. in press.

Foster, S.F., Allan, G.S., Martin, P., Robertson, I.D., Malik, R., 2004b. Twenty five cases of feline bronchial disease (1995–2000). JFMS. in press.

Foster, S.F., Martin, P., Davis, W., Allan, G.S., Mitchell, D.H., Malik, R., 1999. Chronic pneumonia caused by *Mycobacterium thermoresistibile* in a cat. J Sm Anim Pract 40, 433–438.

Foster, S.F., Barrs, V.R., Martin, P., Malik, R., 1998. Pneumonia associated with *Mycoplasma* spp. in three cats. Aust Vet J 76, 460–464.

Gerds-Grogan, S., Dayrell-Hart, B., 1997. Feline cryptococcosis: a retrospective evaluation. J Am Anim Hosp Assoc 33, 118–122.

Gil, J.C., Cedillo, R.L., Mayagoitia, B.G., Paz, M.D., 1993. Isolation of *Mycoplasma pneumoniae* from asthmatic patients. Annals of Allergy 70, 23–25.

Greene, C.E., 1998. Respiratory infections, in: Greene, C.E. (Ed.), Infectious Diseases of the Dog and Cat. Philadelphia. Saunders, pp. 582–594.

Gruffydd-Jones, T.J., 1991. Mycoplasma, in: Proceedings of a Course in Feline Medicine. New Zealand. Massey University, pp. 21–22.

Hawkins, E.C., Kennedy-Stoskopf, S., Levy, J., Meuten, D.J., Cullins, L., DeNicola, D., Tompkins, W.A.F., Tompkins, M.B., 1994. Cytologic characterization of bronchoalveolar lavage fluid collected through an endotracheal tube in cats. Am J Vet Res 55, 795–802.

Hawkins, E.C., Kennedy-Stoskopf, S., Levy, J., Meuten, D.J., Cullins, L., Tompkins, W.A.F., Tompkins, M.B., 1996. Effect of FIV infection on lung inflammatory cell populations recovered by bronchoalveolar lavage. Vet Immunol and Immunopathol 51, 21–28.

Hawkins, E.C., 2000. Pulmonary parenchymal diseases, in: Ettinger, S.J., Feldman, E.C. (Eds.), Textbook of Veterinary Internal Medicine, fifth ed. Philadelphia. Saunders, pp. 1061–1091.

Henik, R.A., Yeager, A.E., 1994. Bronchopulmonary diseases, in: Sherding, R.G. (Ed.), The Cat: Diseases and Clinical Management, second ed. New York, Churchill Livingstone, pp. 979–1052.

Kondo, S., Ito, M., Saito, M., Sugimori, M., Watanabe, H., 1994. Progressive bronchial obstruction during the acute stage of respiratory tract infection in asthmatic children. Chest 106, 100–104.

Kraft, M., Cassell, G.H., Henson, J.E., Watson, H., Williamson, J., Marmion, B.P., Gaydos, C.A., Martin, R.J., 1998. Detection of *Mycoplasma pneumoniae* in the airways of adults with chronic asthma. Am J Respir Crit Care Med 158, 998–1001.

Malik, R., Jacobs, G.J., Love, D.N., 2001. Cryptococcosis: new perspectives on etiology, pathogenesis, diagnosis, and clinical management, in: August, J.R. (Ed.), Consultations in Feline Medicine 4. Philadelphia. Saunders, pp. 39–50.

Malik, R., Wigney, D.I., Muir, D.B., Gregory, D.J., Love, D.N., 1992. Cryptococcosis in cats: clinical and mycological assessment of 29 cases and evaluation of treatment using orally administered Fluconazole. J Med Vet Mycol 30, 133–144.

McCarthy, G., Quinn, P.J., 1986. The development of lavage procedures for the upper and lower respiratory tract of the cat. Irish Vet J 40, 6–9.

Medleau, L., Jacobs, G.J., Marks, M.A., 1995. Itraconazole for the treatment of cryptococcosis in cats. J Vet Intern Med 9, 39–42.

Micillo, E., Bianco, A., D'Auria, D., Mazzarella, G., Abbate, G.F., 2000. Respiratory infections and asthma. Allergy 55(Suppl 61), 42–45.

Mitchell, D.H., Sorrell, T.C., Allworth, A.M., Heath, C.H., McGregor, A.R., Papanaoum, K., Richards, M.J., Gottlieb, T., 1995. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. Clin Infect Dis 20, 611–616.

Moise, N.S., Blue, J., 1983. Bronchial washings in the cat: procedure and cytologic evaluation. Compend Cont Ed 5, 621–628.

Moise, N.S., Wiedenkeller, D., Yeager, A.E., Blue, J.T., Scarlett, J., 1989. Clinical, radiographic, and bronchial cytologic features of cats with bronchial disease: 65 cases (1980–1986). J Am Vet Med Assoc 194, 1467–1473.

Norris, C.R., Griffey, S.M., Samii, V.F., Christopher, M.M., Mellema, M.S., 2002. Thoracic radiography, bronchoalveolar lavage cytopathology and pulmonary parenchymal histopathology: a comparison of diagnostic results in 11 cats. J Am Anim Hosp Assoc 38, 337–345.

Padrid, P.A., Feldman, B.F., Funk, K., Samitz, E.M., Reil, D., Cross, C.E., 1991. Cytologic, microbiologic, and biochemical analysis of bronchoalveolar lavage fluid obtained from 24 healthy cats. Am J Vet Res 52, 1300–1307.

Padrid, P., 2000. Pulmonary diagnostics. Vet Clin N Am Small Anim Pract 30, 1187–1205.

Pedersen, N.C., 1988, in: Feline Infectious Diseases. California. American Veterinary Publications, pp. 295–329.

Peeters, D.E., McKiernan, B.C., Weisiger, R.M., Schaeffer, D.J., Clercx, C., 2000. Quantitative bacterial cultures and cytological examination of bronchoalveolar lavage specimens in dogs. J Vet Intern Med 14, 534–541.

Petrovsky, T., 1990. *Mycoplasma pneumoniae* infection and post-infection asthma. Med J Aust 152, 391.

Randolph, J.F., Moise, N.S., Scarlett, J.M., Shin, S.J., Blue, J.T., Corbett, J.R., 1993. Prevalence of mycoplasmal and ureaplasmal recovery from tracheobronchial lavages and of mycoplasmal recovery from pharyngeal swab specimens in cats with or without pulmonary disease. Am J Vet Res 54, 897–900.

Sabato, A.R., Martin, A.J., Marmion, B.P., Kok, T.W., Cooper, D.M., 1984. *Mycoplasma pneumoniae*: acute illness, antibiotics and subsequent pulmonary function. Archives of Disease in Childhood 59, 1034–1037.

Seggev, J.S., Lis, I., Siman-Tov, R., Gutman, R., Abu-Samara, H., Schey, G., Naot, Y., 1986. *Mycoplasma pneumoniae* is a frequent cause of exacerbation of bronchial asthma in adults. Annals of Allergy 57, 263–265.

Seggev, J.S., Sedmak, G.V., Kurup, V.P., 1996. Isotype-specific antibody responses to acute *Mycoplasma pneumoniae* infection. Annals of Allergy, Asthma & Immunology 77, 67–73.

Simard, M., Dube, P., 1992. L'asthma félin. Le Médecin Vétérinaire du Quebec 22, 129.

Speed, B., Dunt, D., 1995. Clinical and host differences between infections with two varieties of *Cryptococcus neoformans*. Clin Infect Dis 21, 28–34.

Teo, J., Vellayappan, K., Yip, W.C.L., Doraisingham, S., 1986. Mycoplasma pneumoniae and viral infections in childhood asthma. Journal of Tropical Pediatrics 32, 87–89.

Yano, T., Ichikawa, Y., Komatu, S., Arai, S., Oizumi, K., 1994. Association of *Mycoplasma pneumoniae* antigen with initial onset of bronchial asthma. Am J Respir Crit Care 149, 1348–1353.