RESEARCH COMMUNICATIONS II: Pathophysiology, diagnostics, and sampling conditions in animal models and clinics

RECOMBINANT HUMAN VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) PRETREATMENT REDUCES REPLICATION OF BOVINE RESPIRATORY SYNCYTIAL VIRUS (BRSV) AND NEUTROPHILIC AIRWAY EXUDATE IN NEONATAL LAMBS

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Vascular endothelial growth factor (VEGF) promotes angiogenesis and is increasingly recognized as a perinatal regulator of lung maturation and surfactant protein expression. Surfactant proteins A and D have antimicrobial activity against pulmonary pathogens including respiratory syncytial virus (RSV) and perinatal infants are at increased risk for severe manifestations of respiratory syncytial virus (RSV) infection. It was our hypothesis that VEGF therapy would diminish RSV infection in the perinatal lamb model. Newborn lambs were pretreated with VEGF, betamethasone or saline, and following acclimation, inoculated with bovine RSV or sterile media. Tissues were collected at five days post-inoculation which corresponds to the initiation of severe lesions and peak viral replication. Pretreatment with VEGF increased sheep beta defensin 1 mRNA expression, but did not alter surfactant proteins A and D mRNA levels. In RSV-infected lambs, VEGF therapy increased the mean daily body temperature, decreased airway neutrophil exudate and reduced RSV replication. Furthermore, RSV infection increased SP-A mRNA expression and reduced SP-D mRNA expression; however, these RSV-induced alterations were significantly suppressed by VEGF and betamethasone therapy. This study demonstrates novel anti-RSV activity by VEGF therapy and suggests SBD1 may be involved mechanistically.

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OPTIMIZATION OF REPEATED BRONCHOALVEOLAR LAVAGE IN RABBITS

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Background. Bronchoalveolar lavage (BAL) is a relatively non-invasive technique used to obtain diagnostic samples from the lower airways of companion animals with respiratory disease. BAL is also commonly used in laboratory animals to assess pulmonary changes after exposure to air pollutants. However, in experimental studies, BAL is typically performed as a terminal procedure, using large volumes of instillate (25-35 mL/ kg), thus allowing consistent (often > 70%) recovery, and quantitative assessment of BAL fluid cellular, microbiological culture, and biochemical indices.

Study. Herein, we refined BAL in laboratory rabbits so that it could be used as a recoverable procedure, thus allowing repeated assessment of the same subject throughout a chronic air pollutant exposure study. In a cross-over manner, NZW rabbits (n=6; 2.7 – 3.2 kg) underwent BAL using either: (a) 5-mL aliquots of saline (LOW-VOL) pooled samples, or (b) a single 10 mL/kg saline (HIGH-VOL) sample. Results of the LOW- and HIGH-VOL procedures were compared to a terminal BAL procedure using 25 mL/kg. Rabbits were anesthetized with ketamine, midazolam and isoflurane, transorally intubated, and oxygenated for 5 minutes prior to and after BAL fluid collection. Animals were evaluated immediately post-BAL via pulse oximetry and end-tidal capnography. A pharyngeal swab and aliquots of BAL fluid from the first and final procedure were cultured for aerobic isolates. Serology for CAR Bacillus antibody was submitted.

Results. All rabbits recovered uneventfully from either the LOW- or HIGH-VOL BAL procedures. Recovery time was more a function of the difficulty of intubation rather than the BAL volume instilled. No bacterial growth was isolated from BAL fluid samples while the swab grew *Enterococcus faecalis*. All serum samples were negative for CAR Bacillus antibody. The main disadvantage of the LOW-VOL method was that sample recovery was less predictable. On the other hand, although the HIGH-VOL method provided more consistent fluid recovery, certain biochemical indices fell below detectable limits due to greater dilution of epithelial lining fluid (ELF). Using serum and BAL fluid concentrations of urea and albumin, estimates of ELF volume were compared for the three instillation volumes used.

Conclusion. With appropriate pre- and post-lavage care, repeated recovery BAL is possible in rabbits.

(This abstract does not reflect US EPA policy).

EFFECTS OF SAMPLING METHOD ON BACTERIAL GROWTH IN BALF SAMPLES FROM HEALTHY DOGS

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Bronchoalveolar lavage fluid (BALF) samples for microbiologic examination obtained by endoscopy may be affected by oral contamination, resulting in erroneous detection of increased numbers of bacteria caused by oropharyngeal microbes. The purpose of the study was to evaluate the influence of the sampling method determined by 2 different techniques of oxygen supply on the quantitative and qualitative microbial content of endoscopically obtained BALF samples in healthy dogs. We hypothetized that upper airway protection by establishing a laryngeal mask airway might prevent contamination of the BALF samples from contact of the endoscope with the oropharyngeal mucosa, thereby resulting in significantly lower numbers of microorganisms recovered in BALF samples.
Ten healthy Beagle dogs (mean age 1.9 years, mean body weight 16.7 kg) were randomly assigned to group A or B. Both groups underwent endoscopic bronchoalveolar lavage (BAL) on two different occasions 4 weeks apart. Dogs received intravenous (IV) butorphanol (0.4 mg/kg) and a combination of midazolam (0.2 mg/kg) and ketamine (5 mg/kg) IV for induction. Anaesthesia was maintained with continuous rate infusions of propofol (15 mg/kg/h) and butorphanol (0.4 mg/kg/h). Dogs were breathing spontaneously throughout the procedure. BALF was obtained by injection of 1 ml/kg isotonic NaCl twice into an occluded bronchus of the right and left lung through the endoscopic working channel, and lavage fluid retrieval by gentle vacuum pump suction after each injection. BALF from the right and left lungs was sampled and analyzed separately. In group A, the first BAL was performed through the open unprotected oropharynx with additional oxygen supply via a nasal oxygen catheter (NOC), whereas in group B a sterile laryngeal mask airway (LMA) was established for oxygen supply, through which the endoscope was introduced into the trachea. For the second BAL 4 weeks later, groups were switched (group A dogs - LMA, group B - NOC). Raw uncentrifuged BALF samples were serially diluted and quantitatively cultured for bacteria and yeasts on sheep blood 5% (v/v) agar, McConkey agar and Saboroud dextrose agar. After incubation for 72 hours, the colonies were counted, and the number of colony forming units (CFU) per ml BALF was calculated. For microbial identification conventional bacteriological tests and commercial miniaturized identification systems were used. Nucleated cells in BALF were counted and differentiated microscopically. The Kolmogorov-Smirnov test was used to check for normal distribution of the data. Testing for differences in CFU between the two methods (LMA vs. NOS) and influence of repeated BALF sampling was performed with ANOVA on the log transformed CFU data.

For the LMA, the mean±SD CFU/ml BALF was 35610±35230 for the right side (RL) and 37810±50062 for the left side (LL). For the NOC technique, 51758±75585 CFU in RL and 16060±15523 CFU in LL were detected. Nucleated cell counts were 691.0±181.6 (RL) and 734.0±171.6 (LL) for LMA, and 772.0±251.0 (RL) and 748±163.2 (LL) for NOC.
Statistical analysis revealed that there was no significant difference in CFU between the two methods (LMA vs. NOC, $p>0.5$). Also, the diversity of bacterial species cultured did not differ significantly between both methods, whereas we detected a greater diversity in bacterial species on the first BALF samples compared to the second set after 4 weeks,

regardless of the method used. Furthermore, there was no difference in nucleated cell counts between both methods. There was reasonably good association of the cell counts and the log CFU between right and left airways. Analysis further revealed that repeated sampling may have a significant effect on CFU counts. In the second set of experiments, regardless of the method used (LMA vs. NOC), a significant ($p<0.005$) higher number of bacteria was detected.

In conclusion we found that protection of the oral cavity and upper airway with a laryngeal mask does not influence the number of bacteria cultured in BALF samples. These findings indicate, that with careful introduction of the endoscope through an unprotected oropharynx there may be only negligible contamination of the sample. Alternatively, also the application of the laryngeal mask may add to some contamination at the distal mask opening. With the finding of a significant higher number of bacteria in the second experimental set, regardless of the method used (LMA vs. NOC), an effect of the previous sampling on bacterial colonization of the airways can not be ignored.

BACTERIAL SPECIES IN THE RESPIRATORY TRACT OF DOGS WITH RESPIRATORY DISEASE – 800 CASES

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Introduction: The canine respiratory tract (RT) is not a sterile organ, thus, a multitude of different bacterial organisms can be isolated from the upper and lower RT of healthy dogs. Bacterial infection of the RT can either be caused by primary bacterial pathogens such as *Bordetella bronchiseptica* or by the normal bacterial inhabitants, if respiratory defence mechanisms are impaired.

Purpose of the study: The goal of the present study was to investigate the bacterial distribution in the RT of dogs presenting with respiratory disease and to evaluate bacterial sensitivity towards different antibiotics.

Material and Methods: Included in the retrospective study were 800 dogs presented with respiratory signs. Data was collected from the medical records. Excluded were patients with a diagnosis of heart disease.

Results: Median age of the patients was 6.3 years. Of these dogs 53.9 % were male and 46.1 % were female. In these 800 patients 1250 bacterial cultures were taken from different sites of the RT; 751 cultures from the upper (nose, tonsils, larynx), and 499 cultures from the lower (trachea and bronchoalveolar lavage) RT. Negative culture results counted for 5.2 % and 22.4 % of samples in the upper and lower RT, respectively. In the upper RT the bacteria isolated most frequently were *Staphylococcus intermedius* (in 19.0 % of samples), *E. coli* (9.3 %), α-hemolyzing *Streptococcus* (7.7 %), and *Pasteurella multocida* (5.0 %). In the lower respiratory tract the most prevalent bacterial species were α-hemolyzing *Streptococcus* (in 9.0 % of samples), *E. coli* (8.6 %), *Staphylococcus intermedius* (6.8 %), and *Pasteurella multocida* (4.4 %). *Bordetella bronchiseptica*, which can act as a primary respiratory pathogen in dogs, was cultured in 1.5 % and 2.6 % of samples from the upper and lower RT, respectively. The best antimicrobial activity against the bacteria cultured from upper and lower RT was demonstrated for enrofloxacin and amoxicillin-clavulanic acid with the exception of *E.coli*, which showed resistance against each antibiotic drug tested in more than 20 % of cases.

Summary and Conclusions: The study demonstrates that most bacterial species isolated from the RT of dogs with respiratory diseases do not represent bacteria considered primary pathogens, thus probably originating from the normal bacterial microflora. Therefore it can be concluded, that bacterial cultures from the upper respiratory tract of dogs are of little diagnostic significance and can not be recommended as part of the routine work-up in upper respiratory cases. To interpret clinical significance of bacterial culture results from the lower airways additional diagnostic tools, such as cytology, might be included in the work-up to assess grade and significance of bacterial infection.
If antimicrobial therapy is initiated in a patient with respiratory disease before culture and sensitivity test results are available, enrofloxacin or amoxicillin-clavulanic acid can be recommended as first line antibiotics with a favourable pattern of resistance.

DIAGNOSIS OF SINO-NASAL ASPERGILLOSIS: IS QUANTIFICATION OF *ASPERGILLUS* DNA A USEFUL TECHNIQUE?

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The diagnosis of canine sino-nasal aspergillosis (SNA) relies upon the direct visualization of fungal plaques during rhinoscopy/sinusoscopy. Other less invasive methods of diagnosis (CT-scan, serology, cytology, fungal culture) have been proposed but none have proved totally satisfactory. In human medicine, the quantification of fungal DNA has proved valuable in the diagnosis of fungal rhinosinusitis. The objective of the present study was to evaluate the value of tissue and blood *Aspergillus* DNA quantification in the diagnosis of canine SNA.

Fifteen dogs with endoscopically confirmed and untreated SNA and 34 dogs without SNA (13 with a nasal tumour [TUM], 8 with idiopathic lymphoplasmacytic rhinitis [LPR] and 13 without nasal disease [CONT]) were included in the present study. In each dog, one mucosal biopsy sample and 2 ml of blood (in EDTA) were taken and snap-frozen in liquid nitrogen.

Genomic DNA was extracted from 200µl of EDTA blood and the nasal mucosal biopsies (Macherey-Nagel Nucleospin kit) and real-time PCR performed on each sample using Qiagen HotStarTaq Master Mix and a Bio-Rad iCycler iQ. The amount of fungal DNA in the samples was quantified with both an assay to detect *Aspergillus* and *Penicillum* species (PenAsp assay) and species-specific *Aspergillus* assays (for *A. fumigatus*, *A. terreus*, *A. niger* and *A. flavus*). Results were normalised using a canine G3PDH assay to control for variations in sample input and extraction efficiency. A relative copy number for each sample was calculated using the delta Ct method and this value was used as the basis for comparison between the groups.

For each sample, relative copy number of fungal DNA was assessed by quantitative PCR by using a general assay detecting DNA from *Aspergillus* and *Penicillum* species (PenAsp assay). *Aspergillus* species specific assays (for *A. fumigatus*, *A. terreus*, *A. niger* and *A. flavus*) were then run on the samples that yielded a positive result with the PenAsp assay. For the tissue and blood samples, expression differences among the 4 groups of dogs (SNA, TUM, LPR and CONT) were assessed using the Kruskal Wallis test ($P<0.05$). Significant group differences were further assessed using the Mann-Whitney test.

Fungal DNA was detected in all nasal biopsy samples using the PenAsp assay. Although the relative copy numbers overlapped between the 4 groups, there was significantly more fungal DNA in dogs with SNA than in the three other groups. There was significantly less fungal DNA in nasal biopsies from the TUM group than in the CONT and LPR groups. Using the species-specific assays, there were no positive *A. terreus*, *A. niger* or *A. flavus* results in the nasal biopsies. However, *A. fumigatus* DNA was detected in 8 biopsies from dogs with SNA and in 1 biopsy from a dog with nasal tumour.

Fungal DNA was detected in the blood from all control dogs, 3/8 dogs with LPR, 10/13 dogs with nasal tumour and 12/15 dogs with SNA by the PenAsp assay. No statistical difference could be found among the groups. *A. fumigatus* DNA was detected in the blood from 3 dogs with SNA, 9 dogs with nasal tumour, 3 dogs with LPR and 4 control animals. *A. niger* and *A. terreus* DNA was detected in the blood from 2 control dogs and 1 dog with LPR.

The present study shows that fungal DNA is found in greater amount in the nasal mucosa of dogs with SNA than in dogs suffering from other nasal disease and control dogs. There were only *A. fumigatus* positive results in the biopsies from dogs with SNA, confirming that most cases are caused by this species in the dog. The reason for the smaller amount of fungal DNA found in the nasal mucosa of dogs with nasal tumour as compared with that found in the mucosa of control dogs and dogs with LPR is unclear. Finally, the detection of fungal DNA in the blood does not appear to be helpful in the diagnosis of SNA in the dog.

INVESTIGATION OF AIRWAY REACTIVITY BY BAROMETRIC WHOLE BODY PLETHYSMOGRAPHY IN CATS WITH SPONTANEOUS BRONCHIAL DISEASE

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Considerable research has been performed in the last years investigating experimental models of feline bronchitis. The aim of the present study was to investigate airway reactivity in cats presented with naturally occurring bronchial disease. Ten cats were enrolled in the study. For each of them, clinical findings were scored as follows. A cumulative clinical score resulted from a score for cough (0, 1-less than once a week, 2-several times a week, 3 several times a day) and dyspnoea (0 to 2). A radiographic score varied from 0 to 3 (light (1), moderate (2) or severe (3) - bronchial, interstitial, or bronchointerstitial patterns. Bronchoalveolar lavage fluid (BALF) cytology was classified as predominantly macrophagic, predominantly eosinophilic, predominantly neutrophilic, or mixt neutrophilic/eosinophilic. In all cats, basal whole body barometric plethysmography (BWBP) and carbachol bronchoprovocative test (BT) were performed. Considered WBBP parameters included respiratory rate = RR, tidal volume = TV, peak inspiratory and expiratory flows = PIF and PEF. For the BT, BWBP variables were obtained during 5 minutes periods before and after 1-minute of nebulization of sterile saline solution and of up to 6 increasing concentrations of carbachol (0.005 to 0.08%) until Penh, a unitless parameter used as an index of bronchoconstriction, exceeded 300% of baseline. The provocative concentrations of carbachol that increased Penh to 300% of baseline (C-Penh300) were obtained from interpolation from the dose-response curve and was used as airways reactivity indices. In five cats, clinical and radiographical scores were reassessed after 3 weeks and basal BWBP and BT were repeated. Two cats were treated with oral prednisolone (1 mg/kg bid for one week, same dose sid for one week, same dose every other day for the third week), and three others with flixotide (one 250µg puff bid). One of the cat treated with flixotide was reassessed 3 months later. Values of C-Penh300 obtained in cats with bronchitis were compared to values obtained in a group of 13 age-matched healthy cats using an anova test ($P < 0.05$); other values obtained in cats with bronchitis before and after treatment were compared by means of an ANOVA test for repeated measures ($P < 0.05$).

At the time of diagnosis, mean clinical and radiographical scores ($n = 10$) were 2.5 ± 0.4 and 1.9 ± 0.3, respectively (mean ± SEM); C-Penh300 (0.0135 ± 0.002) was significantly lower than in healthy cats.(0.043 ± 0.004). BALF cytology was macrophagic in 4 cats, eosinophilic in 3, neutrophilic in 2, mixt in 1cat. Three weeks after treatment, clinical and radiographical scores, as well as MV, PEF and PIF were decreased, and C-Penh300 increased, although the differences could not be proven to be significant (table 1). However, C-Penh300 remained significantly higher than in healthy cats. In the cat reassessed after 3 months, TV, MV, PEF and PIFcontinued to decrease while C-Penh was still increasing (0.006; 0.007 and 0.009, before, after 3 weeks and 3 months of treatment, respectively).

Clinical, radiographical scores and BWBP values (mean ± SEM) in 5 cats with bronchitis before and after 3 weeks of treatment

	Clinical score	Radio-graphical score	RR	TV	MV	PEF	PIF	C-Penh300
Before	3.1 ±0.6	2.0 ±0.4	52.8 ±2.8	52.7 ±11.2	2653.5 ±476	202 ±112	194 ±56.6	0.007 ±0.0017
After	1.5 ±0.5	1.5 ±0.2	54.8 ±5.6	28.6 ±6.5	1411.7 ±238.7	53.5 ±6	89.6 ±14.4	0.008 ±0.0017

These results show that feline bronchitis is associated with an increased bronchial reactivity. BWBP and BT is a promising non invasive method that could be used for diagnosis and monitoring of the response to therapy in cats with naturally occurring bronchial disease. Future studies on a larger number of cats are needed to to investigate possible correlations between airway reactivity, diagnostic findings and response to therapy.

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BRONCHOPROTECTIVE EFFECT OF INHALED SALMETEROL, SALBUTAMOL AND IPRATROPIUM BROMIDE USING DIFFERENT DEVICES ON MUSCARINIC BRONCHOCONSTRICTION IN HEALTHY CATS

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In human patients suffering from asthma or chronic obstructive pulmonary disease, aerosolisation is a well-established route of delivery for bronchodilators and anti-inflammatory drugs. Not only does inhaled therapy allow reaching higher drug concentrations in target tissues, but it also provides a safer, faster and/or sustained therapeutic response of selected compounds compared to systemic administration. Indications for aerosoltherapy in small animals include asthma, chronic bronchial disease and infections of both lower and upper airways. However, controlled trials on the use of inhaled bronchodilators (BD) in healthy or diseased animals are lacking in feline medicine. The aims of the present study were: 1) to compare the duration of action and efficiency of salmeterol (SLM), salbutamol (SAL), ipratropium bromide (IB) and the combination SAL/IB delivered by a metered dose inhaler (MDI) and/or nebulisation (NEB) in healthy cats with induced bronchoconstriction; 2) to investigate the gain in efficiency by a two- or four-fold increase of drug dosages.

METHODS: Twelve cats aged 18 months (6 neutered males, 6 neutered females) were enrolled in the study. Among BD of interest, two were delivered by an ultrasonic nebuliser (SAL, IB) whereas four (SLM, SAL, IB, SAL/IB) were administrated by a MDI connected to a space chamber and a face mask. Bronchoprotective effect of BD was assessed by barometric whole body plethysmography for unrestrained conscious cats (n = 4 per BD and way of delivery). **Assessment of duration of action and efficiency:** Animals were challenged with increasing concentrations of carbachol (0.005% to 0.5%) 15 min, 1, 2, 4, 8, 24 hours after either SAL 100 µg (1 puff), IB 20 µg (1 puff), nebulised SAL (2.5 mg) or IB (62.5 µg). Bronchial response to long-acting BD SLM 25 µg (1 puff) and combination SAL/IB 100 µg/20µg (each 1 puff) was investigated respectively 2, 4, 8, 12, 24 hours and 15 min, 4, 8, 12, 24 hours after administration. The chosen endpoint was the provocative dose of carbachol increasing Penh, an unitless index of bronchoconstriction, to 300% of baseline value (C-Penh300). One week prior to the first bronchoprotection test, all cats underwent two baseline assessments of airway responsiveness which allowed to define individual basal C-Penh300 value. Changes in airway responsiveness (ΔC-Penh300) expressed as the difference from their respective baseline C-Penh300 values were used to compare efficiency between treatments. **Assessment of drug dosages:** For each BD, at time of maximal effect, a two- or four-fold increment of the initial dose was tested.

RESULTS: **Assessment of duration of action and efficiency:** The maximum calculated C-Penh300 was recorded respectively 15 min after SAL MDI, IB MDI, NEB IB and SAL/IB MDI, 1 hour after NEB SAL and 4 hours after SLM MDI. Maximal effects lasted at least 12 hours for SLM, 4 hours for NEB IB, 15 min for combination SAL/IB and 2 hours for other BD. Basal and post-treatment C-Penh300 values were significantly different up to 24 hours for SLM MDI, 8 hours for SAL MDI and 4 hours for other studied drugs. In terms of efficiency, ΔC-Penh300 calculated at 15 min, 4 hours and 8 hours after

SAL/IB MDI were significantly higher to those obtained for other bronchodilators, which did not differ from each other. Furthermore, they were greater than the sum of the individual effects of SAL MDI and IB MDI. No significant differences between treatments were found when carbachol challenges were conducted at 12 hours and 24 hours after administration. **Assessment of drug dosages:** A four-fold increment of the initial dose of IB MDI, NEB IB, NEB SAL and SLM MDI improved protection of airways against carbachol, leading to significant rises of C-Penh300 values. Despite a four-fold increase of SLM inhaled dose, calculated ΔC-Penh300 values remained lower in comparison to other bronchodilators irrespective to used dosage.

CONCLUSIONS:
These findings suggest

1) SLM has a sustained bronchoprotective effect as long as 12 hours but is the less efficacious drug
2) SAL and IB (NEB or MDI) are short-acting BD with a fast onset of activity (15 min)
3) thanks to independent mechanisms of action, the combination SAL/IB displays a synergistic bronchoprotective effect
4) A four-fold increase of the initial dose of IB MDI, NEB IB, NEB SAL and SLM MDI highly improves the degree of bronchoprotection.

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DIAGNOSTIC IMPACT OF THORACIC RADIOGRAPHY IN AN EXPERIMENTAL MODEL OF FELINE ASTHMA

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Asthma is an underestimated respiratory disease in feline medicine. Thoracic radiography is considered as a helpful complementary examination in diagnosis of feline asthma. The aim of this study was to evaluate, using an experimental model of feline asthma, to which extent thoracic radiography might contribute to assess the severity of the disease.

Material and Methods: Eighteen healthy cats and 12 cats sensitised and exposed to *Ascaris suum* (AS) allergens were investigated on several occasions at the age of 7 to 30 months by thoracic radiography, bronchoscopy and bronchoalveolar lavage (BAL) in order to determine the impact of age and allergen exposures on radiographic results and to relate these results to bronchoscopic findings and bronchoalveolar lavage fluid cytology. Radiographic scores ranging from 0 to 3 evaluating alveolar, bronchial and interstitial pattern were established at several occasions in healthy cats and before and after allergen (AS) challenges in sensitised cats. Bronchoscopy was performed under anaesthesia, a bronchoscopic score ranging from 0 to 3 taking into account mucosal aspect, amount of mucus and bronchial reactivity was established and BAL fluid was sampled. BAL fluid was analysed cytologically. Statistical analysis was performed using a mixed model and Spearman correlation tests.

Results: A significant age-related increase of radiographic score was detected in healthy cats (total score at 7 months: 1.16 ± 0.78; total score at 30 months: 2.35 ± 1.02, $p<0.05$). Within 24 hours after allergen exposure, AS challenge induced a significant increase of the total score (2.9 ± 0.8 at pre-challenge versus 3.9 ± 1.1 at post-challenge, $p<0.05$), which was mainly due to an increase of the interstitial score. Bronchoscopic score significantly increased after AS challenge (0.7 ± 1.2 at pre-challenge versus 2.1 ± 0.8 at post-challenge, $p<0.05$) and BAL cytology revealed a significant increase of eosinophil (3 ± 2 % at pre-challenge versus 30 ± 17 % at post-challenge, $p<0.05$) and neutrophil percentage (9 ± 6 % at pre-challenge versus 25 ± 15 % at post-challenge, $p<0.05$). Four AS challenges performed at 2-month intervals did induce similar changes of the radiographic and bronchoscopic scores and of BAL fluid cytology. Positive and significant correlations were found between radiographic and bronchoscopic scores as well as between BAL fluid eosinophil percentage and radiographic scores.

Conclusion: This investigation has shown that (1) the radiographic score was positively and significantly correlated with animals' age; (2) allergen exposure induced a significant increase of the radiographic score; (3) four consecutive allergen exposures did not induce irreversible changes of the radiographic score; and (4) the radiographic score was positively and significantly correlated with the bronchoscopy score and eosinophil percentage of BAL fluid. In conclusion, these experimental results suggest that thoracic radiography is a sensitive complementary examination for diagnosis of feline asthma.