

COMPARISON OF LUNG ATTENUATION AND HETEROGENEITY BETWEEN CATS WITH EXPERIMENTALLY INDUCED ALLERGIC ASTHMA, NATURALLY OCCURRING ASTHMA AND NORMAL CATS

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Airway remodeling is a prominent feature of feline allergic asthma but requires biopsy for characterization. Computed tomography (CT) has appeal as a minimally invasive diagnostic test. The purpose of this prospective case-control study was to compare indices of airway remodeling between cats with experimentally induced, spontaneous asthma and healthy unaffected cats using CT. We hypothesized that experimental and spontaneous feline asthma would have similar CT airway remodeling characteristics and that these would be significantly different in healthy cats. Experimentally induced asthmatic research cats ($n = 5$), spontaneously asthmatic pet cats ($n = 6$), and healthy research cats ($n = 5$) were scanned unrestrained using a 64-detector row CT scanner. Inspiratory breath-hold CT scans were also performed in experimentally induced asthmatic and healthy cats. Mean $\pm$ extent variation of lung attenuation for each cat was determined using an airway inspector software program and CT images were scored for lung heterogeneity by a board-certified veterinary radiologist who was unaware of cat group status. Groups were compared using one-way ANOVA (unrestrained scans) and the Student's t -test (anesthetized scans) with significance defined as $P < 0.10$. Experimentally asthmatic and spontaneously asthmatic cats had significantly ($P = 0.028$ and $P = 0.073$, respectively) increased lung attenuation compared to healthy cats. Heterogeneity scores were higher in experimentally induced asthmatic cat than in healthy cats. Objective quantification of lung heterogeneity and lung volume did not differ among the three groups ($P = 0.311$, $P = 0.181$, respectively). Findings supported our hypothesis. Inspiratory breath-hold anesthetized CT scans facilitated discrimination between asthmatic and healthy cats in comparison to unrestrained CT scans. © 2015 American College of Veterinary Radiology.

Key words: airway remodeling, asthma, ground-glass opacification, lung attenuation.

Introduction

THE PROGRESSIVE INCREASE in prevalence of allergic asthma in humans as well as in pet cats has led to a need for developing animal models that replicate hallmark features of the disease. Animal models are important to understand mechanistic pathways of disease and to develop novel therapies and treatments. While rodent models of asthma are important to help dissect mechanistic information, they do not spontaneously develop asthma and have important differences in anatomy, physiology, and immunology compared with humans, which limit interpretation of results.¹ A more clinically relevant model of

allergic asthma has been developed in cats, the only animal species that spontaneously develop a syndrome of asthma replicating all the major features of the human disease including airway eosinophilia, airway hyperresponsiveness, and airway remodeling (permanent architectural changes in the lung).¹ This feline model of asthma has allowed for tightly controlled experiments, but extrapolation to the human condition is still limited. Pet cats with spontaneous asthma may also be useful in assessing the analogous condition in human beings, but the feline disease condition (and especially airway remodeling), has not been well characterized.

To date, the major features of asthma—airway eosinophilia, airway hyperresponsiveness, and airway remodeling can be evaluated using cytology of bronchoalveolar lavage (BAL) fluid, ventilator acquired pulmonary mechanics, and histology of lung tissue, respectively.² While lung biopsy is the definitive means to assess for remodeling, it is invasive; thus, computed tomography (CT) has received interest as a noninvasive surrogate diagnostic test. The purpose of this prospective

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Funding Sources: Richard Wallace Research Incentive Grant, University of Missouri, Columbia, MO.

Portions of this study were presented at the Veterinary Research Scholars Program Symposium at Michigan State University.

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Received October 13, 2014; accepted for publication April 23, 2015.
doi: 10.1111/vru.12267

Vet Radiol Ultrasound, Vol. 0, No. 0, 2015, pp 1–7.

case-control study was to compare indices of airway remodeling between cats with experimentally induced and spontaneous asthma and healthy unaffected cats using CT. The results of this study will help determine whether airway remodeling in the research model mimics that of the naturally occurring disease in pet asthmatic cats. While the research asthmatic cat model may be used to better understand the pathophysiology of asthma as well as to design and test novel therapeutics, it is critical that the research model strongly resembles asthmatic pet cats in order for newly designed therapies to translate well into cats with naturally occurring asthma.

Methods

Animals

Three groups of cats were prospectively enrolled in the study: healthy research cats ($n = 5$), research cats with experimentally induced asthma (5), and pet cats with spontaneous asthma (6). Healthy research cats were housed in identical environments as the cats with experimentally induced asthma, had unremarkable physical examinations, did not undergo allergen sensitization, and lacked airway eosinophilia ($<17\%$ eosinophils) as confirmed by BAL cytology. Bermuda grass allergen (BGA) was used to induce experimental feline asthma as previously described.¹ A chronic asthmatic phenotype was maintained using once weekly to twice monthly aerosols of BGA for 9 months after which the cats were evaluated with CT and BAL. Six cats belonging to veterinarians or staff with a chronic history of asthma were recruited prospectively. Clinicopathologic features of these spontaneously asthmatic cats were gathered from a medical records system by a veterinary student (A.B.) at the Veterinary Medical Teaching Hospital, University of Missouri. Information recorded included signalment, clinical signs referable to the respiratory tract, drug therapy, thoracic radiography, ancillary diagnostic tests, and BAL cytology. This study was approved by the University of Missouri Animal Care and Use Committee (ACUC number 6912) and performed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Computed Tomographic (CT) Scans

Scans were performed on each cat using a 64-detector row CT scanner (Aquilion 64, Toshiba America Medical Systems, Tustin, CA). Images of the entire lung field were obtained using 0.5 mm collimation, matrix of 512×512 , pitch of 1.0, small focal spot size, 10–12 cm field of view, 120 kVp and 250 mAs. CT images were reconstructed to 1 mm thickness using a bone algorithm. All cats were scanned with a positioning/restraining device (VetMouseTrap, Universal Medical Systems, Inc.,

Cleveland, OH) that avoids the need for anesthesia. To better understand the discrepancy in results between subjective scoring and objective quantitative assessment of lung heterogeneity using airway inspector freeware (3D Slicer, <http://www.slicer.org/>), healthy and experimentally asthmatic research cats were anesthetized and CT scans repeated. Owners of pet cats declined general anesthesia to perform these CT scans so they were performed in research cats only. A 5-s inspiratory breath-hold technique was used to maximize lung expansion and avoid motion artifact. For these scans, research cats were premedicated with ketamine (30 mg IV); and propofol was used for anesthetic induction (6 mg/kg IV) and maintenance (0.3 mg/kg/min). Cats were mechanically ventilated using a commercial ventilator (Engstrom Carestation, GE Healthcare, Fairfield, CT) with a positive-end expiratory pressure (PEEP) of 2 cmH₂O and 40% fraction of inspired oxygen. The tidal volume set at 10 mL/kg and adjusted at 2.5 mL/kg increments without exceeding 15 mL/kg to ensure an end-tidal CO₂ of 36–40 mmHg. Images were transferred to a dedicated image analysis workstation (Empiric PACS system, Encompass, Fujifilm Medical Systems), and viewed using image analysis freeware (OsiriX, v. 3.9.4, 32 bit, www.osirix-viewer.com). Images were displayed using a lung window (window width (WW) 1600; window level (WL) 550). For each cat, BAL cytology was performed after the CT scans.

Subjective evaluation of lung heterogeneity was performed by an ACVR board-certified veterinary radiologist (I.M.) who was unaware of cat group status. Anonymized CT images for each acquisition were exported as clips (.mov) using image analysis software without loss of resolution. For each generated clip, an alias was created and was entitled a random number between 10 and 100 for the awake CT scans and between 100 and 150 for anesthetized scans using a random number generator (www.randomnumbergenerator.com). A list of the randomized number and corresponding name of the cats and groups was kept hidden until scoring was terminated. Clips generated for each condition were saved in two separate folders and scored by the radiologist. Clips were reviewed using a free media player (Quicktime Player, v. 10.3, Apple Inc., Cupertino, CA) by either playing the clip multiple times or using the frame-by-frame function when deemed necessary. Each clip was evaluated for heterogeneity defined as the presence of at least one area containing a hyperattenuating parenchymal lesion with or without bronchial wall thickening. Clips for which no lesion was observed were scored as 0. Clips with at least one lesion were then scored mild (score = 1), moderate (2), and severe (3) according to the extent of lesions. Within this scoring scheme, cats with lesions found at multiple locations throughout the lungs and occupying a large proportion of the lung field (more than 50% of the lung field) were classified as severe whereas cats with only one hyperattenuating region

occupying less than 10% of the lung field were attributed a score of 1 (mild). Cats with lesions found in a few areas of the lungs and/or occupying between 10% and 50% of the lung field were classified as moderate. Additionally, CT images from awake scans were evaluated for the presence of motion artifact. Motion artifact was characterized by loss of crisp edges when evaluating pulmonary vasculature or airway walls and the lung field as having one or more areas of blurriness. Motion artifact was then classified as mild, moderate, and severe depending on the number of images being affected: less than one-third of the images (mild), between one- and two-thirds of the images (moderate), and more than two-thirds of the images (severe).

Objective assessment included calculation of total lung volume, mean lung attenuation, and extent of variation (an index of lung heterogeneity) of the lung attenuation using an airway inspector software program (3D Slicer, 3D Slicer.org). To perform these measurements, tomographic images (DICOM format) for each cat were imported into the program (A.B.). Lung density histograms were obtained for Hounsfield units (HU) of -300 to -1000 using the COPD-Emphysema module based on density mask analysis of lung parenchyma as described previously.³ Distribution curves were generated for each cat and values for mean and extent of variation of lung attenuation, and lung volume were collected. To account for small variation in attenuation values between scans, lung attenuation (mean and extent of variation) was normalized to the tracheal lumen attenuation in each cat. Attenuation in HU was measured by manually drawing a circular region of interest in the lumen of the trachea from the most cranial aspect of the lung lobes to the tracheal bifurcation on each 1-mm transverse image taken at 10-mm interval. Tracheal lumen attenuation values were then averaged to provide one value to be used for normalization. Total lung volume was normalized to body weight (kg) for each cat.

Statistical Analysis

Data from the three groups were tested by one author (I.M) for normality using a Shapiro–Wilk test (Systat Software, Inc., San Jose, CA). Normally distributed data were analyzed using a one-way analysis of variance (ANOVA) followed by post hoc testing using Holm–Sidak method (heterogeneity scores, motion artifact, mean, and extent of variation). Nonnormally distributed data were analyzed using Kruskal–Wallis ANOVA on ranks. Heterogeneity scores performed on anesthetized cats were analyzed using a Student's *t*-test. Statistical analysis was performed using commercially available software (SigmaPlot 12, Systat Software Inc., San Jose, CA). Given that macroscopic CT changes were compared with microscopic histopathologic changes, statistical significance was set at $P < 0.10$.

Results

Signalment, Body Weight, and BAL Cytology of Research Cats

All research cats were domestic shorthairs and intact males; healthy cats were 2 years of age and asthmatic cats were 1 year old. The group mean $\pm$ SD body weight was 4.5 ± 1.0 for healthy cats and 5.4 ± 1.0 for asthmatic cats. Healthy research cats had group mean $\pm$ SD % BAL eosinophils of $7 \pm 5\%$; whereas experimentally asthmatic cats had $58 \pm 12\%$ eosinophils. Body weights were also similar between all three groups ($P = 0.319$).

Clinicopathologic Features of Spontaneously Asthmatic Pet Cats

Breeds for the spontaneously asthmatic pet cats included DSH ($n = 3$), Ocicat (1), Cornish Rex (1), and Siamese (1). Five cats were female (four spayed, one intact) and one cat was a sexually intact male. Average age was 8.2 years (range 4–14 years) and average body weight was 5.4 kg (range 4.3–6.7 kg). Clinical signs included chronic cough ($n = 6$) and increased respiratory effort ($n = 2$). All of the pet cats were being concurrently treated with oral or inhaled glucocorticoids. Other ancillary treatments in some cats included bronchodilators, anthelmintics, and antibiotics. Thoracic radiographs revealed a bronchointerstitial pattern ($n = 6$), alveolar infiltrate (1), and cardiomegaly (2). Heartworm antibody ($n = 3$) and antigen tests (2) and fecal tests (2) were negative for parasites. Bronchoalveolar lavage cytology revealed eosinophilic inflammation ($n = 6$; group mean $\pm$ SD % BAL eosinophils $50 \pm 30\%$); of these, two were interpreted as mixed inflammation with an eosinophilic component. Bacterial culture of aerobic organisms showed light growth ($n = 3$) in the absence of septic, suppurative inflammation; mycoplasma PCR results were negative ($n = 2$).

Lung Heterogeneity Scoring in Awake Cats

Images of the entire lung field were obtained in each cat without the need for sedation using the VetMousetrap (Universal Medical Systems, Inc., Cleveland, OH). In a few cases, the scan was repeated a second time if motion artifact was thought to decrease the quality of the images the first time. Overall, images were felt to be of adequate diagnostic quality despite mild to moderate motion artifact present in some images (Fig. 1). Subjective evaluation of the images did not reveal any difference in motion artifact between groups ($P = 0.423$). One-way analysis of variance demonstrated a significant difference in heterogeneity score among the three groups of cats ($P = 0.056$). Post hoc testing revealed that cats with experimentally induced asthma had a significantly higher heterogeneity score than healthy

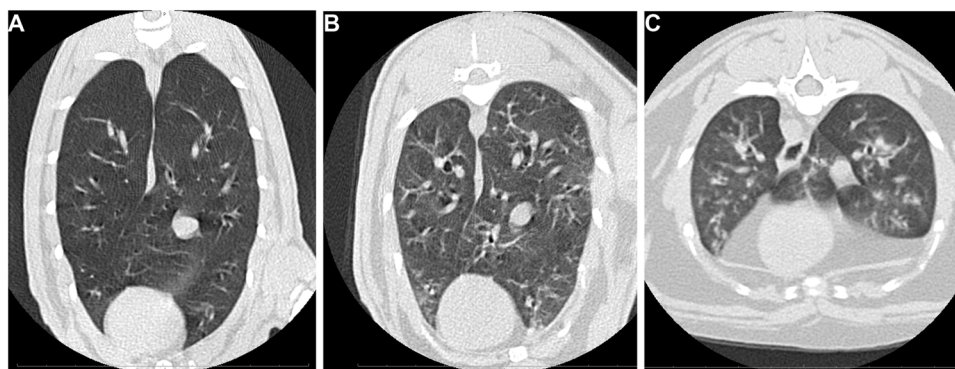


FIG. 1. CT transverse images of a healthy cat (A), experimentally induced asthmatic cat (B), and spontaneously asthmatic pet cat (C) taken at the level of the caudal and accessory lung lobes. Images were deemed to be of diagnostic quality despite mild motion artifact. Healthy cat lungs have lower attenuation values in Hounsfield units (HU) resulting in their darker appearance when compared to the experimentally induced and spontaneously asthmatic cats. On visual inspection, lungs of healthy cats appear more homogenous than those of experimentally induced asthmatic cats. There was no preferential localization of lung parenchymal lesions in either experimentally induced or naturally occurring asthma.

research cats (mean $\pm$ SD experimentally asthmatic cats 1.8 ± 1.1 , healthy cats 0.2 ± 0.4 ; $P = 0.075$) but no significant difference between pet asthmatic cats (mean $\pm$ SD 1.5 ± 1.2) and either research group of cats ($P = 0.63$, 0.10 , respectively). Examples of images from cats with heterogeneity scores of 0, 1, 2, and 3 are illustrated in Fig. 2. Experimentally asthmatic and spontaneously asthmatic cats had lesions observed in varying lung lobes throughout the entire lung field without evidence of site predilection. Asthmatic cats tended to have increased bronchial wall thickness that was reflected by an increased number of visible small bronchi (third generation) (Fig. 2D). In addition, one cat with naturally occurring asthma had complete consolidation of its right middle lung lobe as shown in Fig. 3. An attenuation value of 29.621 HU was obtained from a region of interest drawn over the consolidated lung.

Objective Assessment of Mean Lung Attenuation, Extent of Variation of Lung Attenuation, and Total Lung Volume

There was a significant difference in mean lung attenuation among the three groups of cats (group mean $\pm$ SEM: healthy cats -865.5 ± 34.4 HU; experimentally asthmatic cats -738.1 ± 20.1 HU; pet asthmatic cats -772.2 ± 29.1 HU; $P = 0.025$). As shown in representative scans from individual cats in 2, post hoc testing demonstrated that healthy cats (Fig. 2A) had significantly lower mean lung attenuation than experimentally asthmatic (Fig. 2C) and spontaneously asthmatic cats (Fig. 2B, D) ($P = 0.028$ and $P = 0.073$, respectively). There was no significant difference in the objective degree of heterogeneity of lung attenuation between the groups of cats (group mean $\pm$ SEM: healthy cats 130.2 ± 12.6 HU; experimentally asthmatic cats 145.3 ± 1.6 HU; pet asthmatic cats 145.0 ± 4.8 HU; $P = 0.311$). No significant difference in lung volume was found between groups (group mean $\pm$ SEM: healthy

cats, 58.1 ± 15.4 mL/kg; experimentally asthmatic cats, 41.0 ± 3.7 ; pet asthmatic cats, 28.6 ± 5.2 ; $P = 0.181$).

Lung Heterogeneity Scoring in Anesthetized Research cats

Using the same criteria for lung heterogeneity scoring as for the awake scans, subjective assessment of CT images from anesthetized animals demonstrated that cats with experimentally induced asthma had significantly higher heterogeneity scores than healthy research cats (mean $\pm$ SD experimentally asthmatic cats 1.8 ± 0.8 , healthy cats 0.3 ± 0.5 ; $P = 0.002$). The conspicuity of the lesions on images from anesthetized asthmatic cats was observed to be superior to images obtained in awake asthmatic cats as shown in a representative cat in Fig. 4. This observation was thought to result from the lack of motion artifact in anesthetized cats. Atelectasis was either completely absent or minimal in all the studies with the inspiratory breath-hold technique.

Discussion

The results of this study supported our hypothesis that cats with naturally occurring asthma share similarities on CT examination with experimentally induced asthmatic cats and that these CT characteristics are different from healthy cats. The first parameter evaluated was lung attenuation, an index of lung parenchymal density that can be quantitatively measured in HU using CT. We have previously demonstrated that asthmatic cats have lesions on CT characterized by parenchymal bands, ground glass opacity (haziness), consolidation and increase bronchial wall thickening.⁴ All of these lesions contribute in asthmatic cats to an increase (more positive) in lung attenuation; the increase being proportional to the severity and extent of the lesions. The lack of concurrent histologic examination

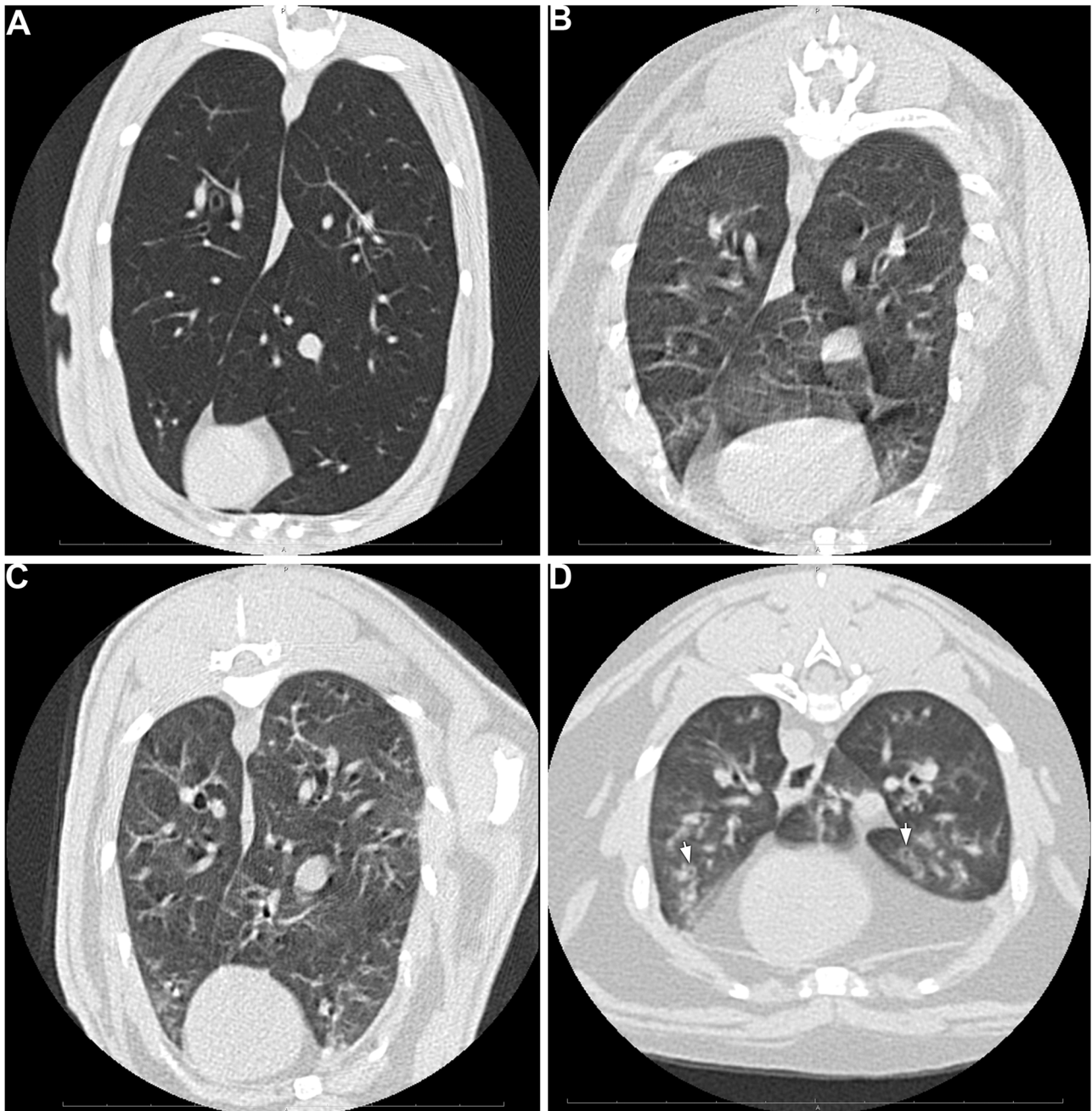


FIG. 2. CT transverse images of cats with heterogeneity scores ranging from 0 (absent) to 3 (severe). (A) healthy cat with a score of 0 for which no lung parenchymal lesion was found. (B) Pet asthmatic cat with mild variation in parenchymal lesions that obtained a score of 1. (C) Experimentally induced asthmatic cat (same cat as in Fig. 1B) with moderate heterogeneity in lung parenchymal lesions for which a score of 2 was attributed. (D) Pet asthmatic cat (same cat as in Fig. 1C) classified as having severe heterogeneity of parenchymal lesions and assigned a score of 3. Interestingly, note the diffuse bronchial wall thickening (white arrows point to some of them in cross section) in the spontaneously asthmatic cat (D).

of lung tissue in the cats of this study to correlate with CT changes is a limitation. However, our laboratory has previously demonstrated histologic evidence of airway remodeling in experimentally asthmatic cats exposed for 1 year to aerosolized allergens both in the proximal and distal airways including epithelial cell hyperplasia, smooth muscle hypertrophy and hyperplasia, submucosal gland

hyperplasia, and peribronchiolar inflammation.¹ Spontaneously asthmatic cats have been described to have similar changes including epithelial erosion, smooth muscle hypertrophy extending from the bronchial walls to the alveolar ducts, hyperplasia of the mucus secreting apparatus, and mixed inflammation (eosinophils, neutrophils, lymphocytes, and plasma cells).^{5,6} Thus, we postulate that the

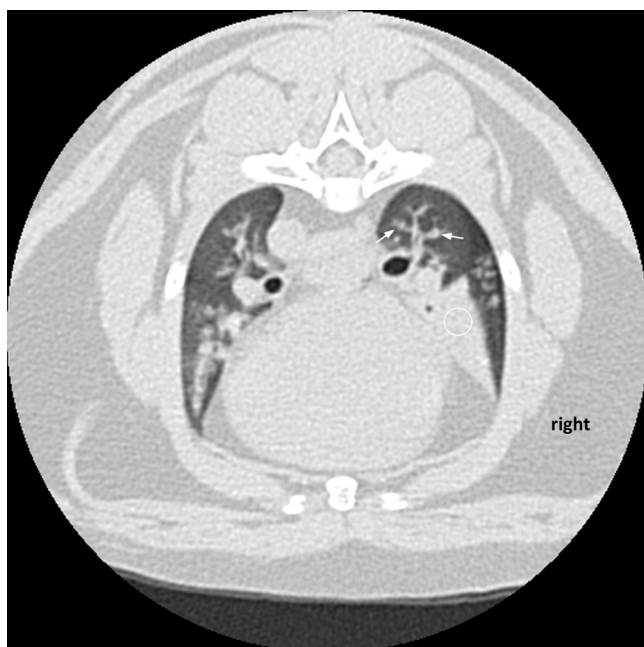


FIG. 3. CT transverse image of a cat with naturally occurring asthma illustrating a complete consolidation of the right middle lung lobe. A small region of interest (white circle) measuring 0.175 cm² was drawn over the right middle lung lobe, which revealed an attenuation value equal to 29 Hounsfield units (HU). The white arrows indicate thickened bronchi in cross section.

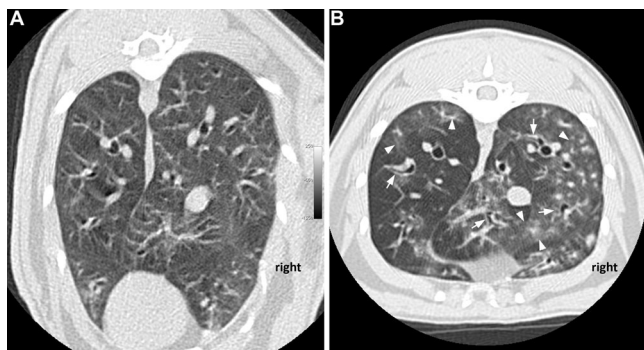


FIG. 4. Experimentally induced asthmatic cat undergoing unrestrained (A) and anesthetized (B) CT scans taken at the level of the caudal and accessory lung lobes. The absence of motion provided by the inspiratory breath-hold technique drastically improves the conspicuity of lung parenchymal lesions consisting of areas of ground glass opacification (arrowheads). In this cat, bronchial and bronchiolar wall thickening (arrows) is more easily appreciated on image obtained with the breath-hold anesthetized technique.

increase in lung attenuation in cats with either experimentally induced or naturally occurring asthma is the result of a combination of these processes.

The awake condition in which the scans were acquired may have contributed to increased lung attenuation in experimentally induced and spontaneous asthmatic cats versus healthy cats. Increased lung inflation with lower attenuation during an anesthetized inspiratory breath hold compared with an awake more shallow, mid-inspiratory or even expiratory breath in the presence of lung lesions

may also have played a role in the calculation of mean lung attenuation between asthmatic and healthy cats. Asthmatic cats are prone to airway collapse due to bronchospasm and airway hyperresponsiveness, airway wall edema or cellular infiltration, and mucus plugging; these would be associated with higher attenuation during exhalation or early inspiration. Inspiratory breath holds, especially with the inclusion of PEEP help to stent open airways, which may otherwise collapse and lead to lower (more negative) attenuation values. Healthy cats would not be expected to have pronounced differences in lung attenuation between full inspiration and various degrees of expiration.

Published “reference ranges” for mean lung attenuation are dependent on the ventilatory technique used to acquire the CT images and thus must be interpreted with caution. Mean lung attenuation measured in our experimentally and spontaneously asthmatic cats was included in the wide range of mean lung attenuation reported in anesthetized healthy cats.⁷ The authors of the aforementioned study attributed the high lung attenuation values obtained using two of the four ventilator protocols to a lower overall aeration of lung tissue. Acquisition of CT images in our study was performed while the cats were awake and breathing room air avoiding confounding factors from ventilatory protocols. This underscores the importance of standardizing the methods of scan acquisition, especially when comparing cats before and after treatment. Since the primary goal of the current study was to compare pet asthmatic cats to experimentally induced asthmatic cats and since anesthesia is often contraindicated in asthmatic cats to avoid further respiratory compromise, it was essential to design a protocol that would be suited for future studies applicable to pet asthmatic cats.

A certain degree of lung heterogeneity on CT images is inherent in healthy animals due to gravitational effects and due to lung volume/phase of respiration. In this investigation, cats were evaluated in the same body position, so variation associated with gravitational effects on ventilation was similar in all groups. Because cats were awake and breathing spontaneously, it was not possible to control for lung volume or phase of respiration although no difference in lung volume was observed between groups. Atelectasis, which consists of a lack of inflation or collapse of a part of a lung lobe or an entire the lung lobe, does not seem to have played a role in the presence of lung heterogeneity as only one cat had atelectasis/consolidation in the right middle lung lobe. Thus, variation in lung density is likely attributable to other factors such as the presence of structural remodeling associated with asthma. Furthermore, the use of 40% fraction of inspired oxygen and a PEEP of 2 cm H₂O contributed to the absence of noticeable atelectasis in the anesthetized cats; these ventilatory conditions likely contributing to maintain the nitrogen stenting effect.

The absence of a significant difference in lung heterogeneity between groups using an automated quantification in comparison to subjective visual scoring may be attributed to several factors. First, the relatively small number of animals per group and the variability of lung heterogeneity, particularly in healthy cats, limit the statistical power of these analyses. Second, upon direct comparison between awake and anesthetized scans it appears that motion artifact, regardless of its severity, plays a crucial role in the quantitative measure of lung attenuation as well as on visual scoring. The presence of motion artifact may increase the difficulty in recognizing healthy cats as having normal lung parenchyma, and may also result in underestimation of number, distribution, and severity of lesions in asthmatic cats.

In conclusion, the results of this pilot study indicated that unrestrained CT scans warrant further study for discriminating asthmatic versus healthy cats. Moreover, cats with experimentally induced or naturally occurring asthma shared similarities with respect to their lung attenuation values and subjective heterogeneity scores. While awake

and unrestrained CTs had the advantage of not requiring anesthesia, motion artifact tended to result in an underestimation of the presence and severity of the parenchymal lesions in asthmatic cats and precluded analysis of other parameters such as bronchial wall thickness that could be obtained from anesthetized CTs. Despite these limitations, awake CT scans may still be valuable in a clinical setting to document lesions associated with asthma or to rule out other causes of respiratory signs (e.g., pleural and cardiac causes associated with secondary respiratory signs, lung nodules or masses, cavitory lesions, etc.) occurring concurrently in previously diagnosed asthmatic cats or in cats of unknown asthmatic status and for which anesthesia is contraindicated. Further studies using CT performed on anesthetized experimental feline asthma “models” may be valuable to serially investigate new therapies targeting lung remodeling.

ACKNOWLEDGMENTS

The authors thank Jan Kunkel and T.J. Stockton for their technical assistance during acquisition of CT images.

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