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AUTHORS: Benjamin N. Nevitt; Jennifer N. Langan; Michael J. Adkesson; Mark A. Mitchell; Margaret Henzler; Randi Drees

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Comparison of air sac volume, lung volume, and lung densities determined by use of computed tomography in conscious and anesthetized Humboldt penguins (*Spheniscus humboldti*) positioned in ventral, dorsal, and right lateral recumbency

Benjamin N. Nevitt, DVM; Jennifer N. Langan, DVM; Michael J. Adkesson, DVM; Mark A. Mitchell, DVM, PhD; Margaret Henzler, MS; Randi Drees, Dr med vet

Objective—To determine the effects of recumbency on air sac volume, lung volume, and lung densities in CT images of healthy, conscious and anesthetized spontaneously breathing Humboldt penguins (*Spheniscus humboldti*).

Animals—25 adult (13 male and 12 female) Humboldt penguins.

Procedures—CT images of conscious penguins in ventral recumbency and anesthetized penguins in dorsal, ventral, and right lateral recumbency were obtained. Air sac volume, lung volume, and lung densities in CT images were calculated. A paired samples *t* test was used to determine whether right and left lung densities differed among recumbencies. Repeated-measures ANOVA (controlled for sex and order of recumbencies during CT) was used to determine whether air sac or lung volumes differed among recumbencies.

Results—Recumbency had a significant effect on air sac volume but not lung volume. Air sac volume was largest in conscious penguins in ventral recumbency (mean ± SD, 347.2 ± 103.1 cm^3) and lowest in anesthetized penguins in dorsal recumbency (median, 202.0 cm^3; 10th to 90th percentile, 129.2 to 280.3 cm^3). Lung densities were highest in anesthetized penguins in dorsal recumbency (right lung median, 0.522 g/cm^3; left lung median, 0.511 g/cm^3) and lowest in anesthetized penguins in ventral recumbency (right lung median, 0.488 g/cm^3; left lung median, 0.482 g/cm^3).

Conclusions and Clinical Relevance—Results indicated that anesthetized Humboldt penguins had the lowest air sac volume and highest lung densities in dorsal recumbency. Therefore, this recumbency may not be recommended. Minimal changes in lung volume were detected among recumbencies or between conscious and anesthetized penguins. (*Am J Vet Res* 2014;75:739–745)

Eleven of the 18 living species of penguins are considered endangered or vulnerable, including Humboldt penguins (*Spheniscus humboldti*),[a] which are protected under the United States Endangered Species Act and appendix I of the Convention on International Trade of Endangered Species international treaty. The closely related African penguin (*Spheniscus demersus*) is listed in appendix II of that international treaty.[b] These 2 species and various other species of penguins are housed in many zoological facilities and aquariums. Maintenance of the health of these animals is important because each bird has a role in conservation and education programs.

Computed tomography is a sensitive diagnostic test that can be used to efficiently evaluate anatomic and pathological features of animals in detail. Although few reports[1–3] of CT for birds have been published, CT seems to be better than radiography for the diagnosis and determination of treatments for such animals with pathological lesions. Respiratory disease, in particular aspergillosis, is one of the most important causes of morbidity and death for captive penguins.[4–14] Determination of a diagnosis of aspergillosis and other respiratory diseases can be difficult by use of radiography because early lesions in the lungs and air sacs may not be detected. Computed tomography has been used to evaluate the anatomic and pathological features of whoop-

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From the Illinois Zoological and Aquatic Animal Residency Program (Nevitt) and Department of Veterinary Clinical Medicine (Langan, Mitchell), College of Veterinary Medicine, University of Illinois, Urbana, IL 61802; Chicago Zoological Society, Brookfield Zoo, 3300 Golf Rd, Brookfield, IL 60513 (Langan, Adkesson); the Department of Human Oncology, University of Wisconsin, Madison, WI 53792 (Henzler); and the Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706 (Drees). Dr. Drees's present address is Department of Clinical Sciences and Services, Royal Veterinary College, University of London, Hertfordshire, AL9 7TA, England.
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Address correspondence to Dr. Nevitt (benjamin.nevitt@yahoo.com).

ing cranes (*Grus americana*) suspected to have respiratory disease attributable to aspergillosis.[c] Computed tomography may have higher sensitivity for detection of small lung and air sac abnormalities than digital radiography for birds[1,15] and has had high diagnostic value in the authors' clinical practice. There is high potential for the use of CT as a diagnostic method for detection of respiratory diseases in penguins, but baseline data regarding anatomy, air sac volumes, lung volumes, and lung densities for clinically normal birds are needed to accurately assess birds with respiratory diseases. To the author's knowledge, no study has determined objective information regarding CT imaging of lungs and air sacs of penguins. Such data may be important for accurate assessment of the respiratory system and determination of severity of respiratory diseases in penguins and other species of birds.

Because of 3-D volume rendering from cross-sectional images, CT allows quantitative estimation of volume. Few studies have been conducted in which air sac volume in birds[16–18] and lung volume in turtles[19] have been determined with CT. Computed tomography has been used to determine pulmonary, renal, and hepatic volume and density in humans and other animals.[20–22]

Avoidance of a long duration of dorsal recumbency during anesthesia for birds has been recommended on the basis of the theory that air sacs can be compressed by internal organs.[23–25] However, ventral recumbency may cause respiratory compromise during anesthesia, as movement of the keel may be restricted by the weight of a bird.[26–28] Anesthetized, spontaneously breathing red-tailed hawks (*Buteo jamaicensis*) have the highest lung and air sac volumes and the lowest lung densities when positioned in ventral recumbency, compared with values for birds in right lateral and dorsal recumbencies.[18] To the author's knowledge, no studies have been conducted to evaluate air sac volume or lung density in clinically normal penguins. Similarly, no studies have been conducted in which those variables were evaluated in various body positions or sedation statuses of such birds. Such information may be useful for the use of CT as an imaging method for the evaluation of penguins with respiratory disease.

The objective of the study reported here was to determine air sac volumes, lung volumes, and lung densities in conscious and anesthetized healthy Humboldt penguins and to evaluate the effect of body position (dorsal, ventral, and right lateral recumbencies) on values of those variables by use of quantitative CT image analysis. The authors hypothesized that air sac volume would vary among positions but that lung volumes and densities would not vary among positions.

Materials and Methods

Animals—The study protocol was approved by the Chicago Zoological Society Institutional Animal Care and Use Committee. Twenty-five adult Humboldt penguins (13 males and 12 females; weight range, 2.8 to 5.1 kg) housed at the Chicago Zoological Society Brookfield Zoo were used in the study. The birds were housed in 2 indoor exhibit areas and fed a diet of whole fish. All birds were determined to be healthy on the basis of results of physical examinations, CBCs, serum biochemical analyses, and an *Aspergillus* test panel (circulating *Aspergillus* antibody titers, galactomannan concentrations, and plasma protein electrophoresis results performed at the University of Miami Avian and Wildlife Laboratory, Miami, Fla). Food was withheld from the birds for at least 12 hours prior to performance of study procedures.

Study procedures—The penguins were randomly assigned to 1 of 3 groups in a prospective study design. Each group differed regarding the order of positioning of birds for CT scans after induction of anesthesia. For group 1 (n = 9), penguins underwent CT in ventral, dorsal, and then right lateral recumbency. For group 2 (n = 8), penguins underwent CT in dorsal, right lateral, and then ventral recumbency. For group 3 (n = 8), penguins underwent CT in right lateral, ventral, and then dorsal recumbency.

The initial CT was performed for conscious penguins in ventral recumbency by restraining the birds in an appropriately sized rubber traffic cone, with the narrow end trimmed and blunted to allow the head to pass through and have full range of motion. Restraint was minimal and had no effect on ventilation of the penguins. Restraint in the traffic cone in ventral recumbency was accomplished by use of foam pads and elastic bands to prevent the birds from backing out of the cone. Narrow linear openings had been cut into the part of the cone at the dorsal aspect of the penguins to allow body heat to escape. Whole-body helical CT was performed. Then, penguins were allowed at least 5 minutes to stand without restraint before induction of anesthesia. During this time, CT images were reviewed to determine whether motion artifact was severe, which required repeated CT. A second conscious CT was required for 2 (8%) birds because of motion artifact.

Anesthesia was induced for each penguin with 5% isoflurane[d] in oxygen administered by face mask. The birds were intubated with a 2.5- to 4.0-mm (depending on body size and tracheal diameter) uncuffed or uninflated cuffed endotracheal tube, and anesthesia was maintained with 2% to 4% isoflurane in oxygen (flow rate, 1 to 1.5 L/min) with spontaneous ventilation. During all CT examinations, respiratory rate, heart rate (determined with a pulse oximeter and auscultation between CT examinations), and cloacal temperature were monitored. A pulse oximeter placed on webbing between toes was used to estimate arterial blood oxyhemoglobin oxygen saturation between recumbencies during anesthesia.

CT imaging—Penguins were positioned for CT within 5 to 10 minutes after induction of anesthesia. Each bird was positioned in the first recumbency position for its group (dorsal, ventral, or right lateral recumbency). Then, whole-body helical CT was performed. After each CT, anesthesia was maintained while the penguins were positioned in an upright, standing position for at least 3 minutes to allow internal organs to return to normal positions and lungs and air sacs to inflate. Then, each bird was positioned in the next recumbencies for its group and CT examinations were repeated for each position. The birds were held in an upright position for 3 minutes between CT examina-

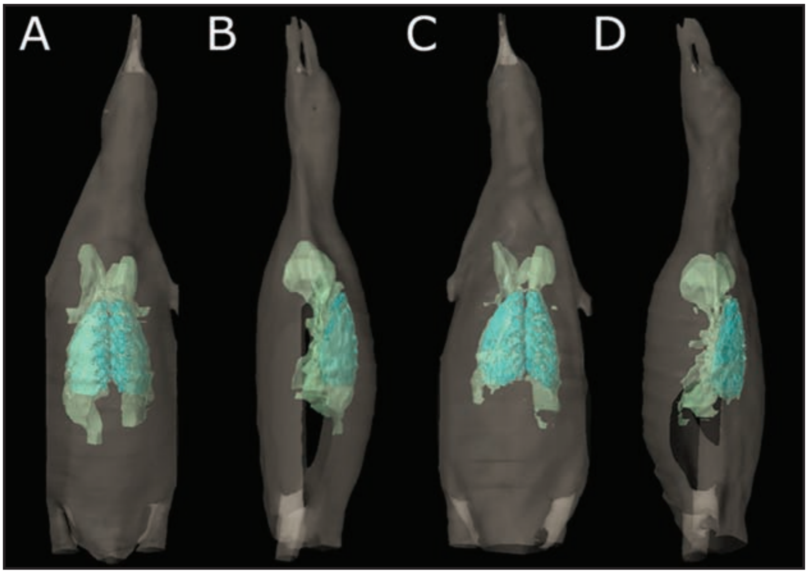

Figure 1—Representative dorsal (A and C) and lateral (B and D) views of 3-D reconstructions of CT images of Humboldt penguins (*Spheniscus humboldti*) in ventral (A and B) and dorsal (C and D) recumbencies. In all images, lungs are highlighted in blue and air sacs are highlighted in yellow. Notice that the air sac volume is higher in penguins in ventral recumbency than it is in penguins in dorsal recumbency.

tions. All CT examinations were completed during a single anesthetic period for each bird for each of the recumbencies.

Computed tomography was performed with a single-slice helical CT scanner[e] (slice thickness, 2 mm; helical acquisition mode; tube rotation time, 1 second; pitch, 1.4; display field-of-view, 15 cm; 120 kVp; and 100 mAs). The CT images were reconstructed with a high–spatial frequency reconstruction algorithm (proprietary term, bone algorithm) without additional filters. Volumetric and density analysis of air sac and lung volumes and lung densities were performed by use of commercial radiation therapy treatment planning software.[f] With this software, volumetric estimates of the lungs and air sacs were calculated and densities of the lungs were estimated. For each CT, a profile density measurement tool was used to evaluate the density range (in Hounsfield units) over a region incorporating lungs and air sacs. A CT density range tool was then used to autocontour the combined lung and air sacs over a specified region on each slice throughout the scan. All air sacs were included in measurements, including cervical air sacs. Primary bronchi were also included in the volume measurements, but the trachea was not included. Areas outside the body were automatically excluded and improperly delineated areas, such as gas in the gastrointestinal tract, were manually erased by the operator. The software then calculated total volume for the combined lungs and air sacs from the contoured areas on each CT image. This process was repeated to autocontour only lung tissue in each CT image. Improperly delineated areas were manually erased by the operator. The software was used to calculate lung volume (including primary bronchi). Volume of air sacs was then determined by subtracting lung volume from combined lung and air sac volume. This process was repeated by a single operator (BNN) for all 4 recumbency image sets for each bird in the study.

Mean density of each lung was measured with an area density tool to determine the mean radiodensity in 3 areas (cranial, middle, and caudal) in each lung and recumbency evaluated. The mean of those 3 measurements was calculated; this was the mean radiodensity (in Hounsfield units) of each lung. The CT scanner was routinely calibrated with a phantom with plugs of known densities to determine the relationship between the measured radiodensity and the tissue density (in grams per cubic centimeter). The 3-D reconstruction of CT images was performed for purposes of subjective evaluation (**Figure 1**).

Statistical analysis—The distribution of data was evaluated with the Shapiro-Wilk test and with skewness, kurtosis, and q-q plots. Normally distributed data were reported as mean ± SD and range. Nonnormally distributed data were reported as median, 10th to 90th percentile, and range. Nonnormally distributed data were log transformed. A paired samples t test was used to determine whether right or left lung densities differed among positions. For univariate analysis, a Student t test was used to determine whether air sac volume, lung volume, or lung densities differed between sexes of birds. Pearson correlation was used to determine whether air sac volume or lung volume were correlated with weight. A general linear model for repeated measures was used to determine whether lung volume, air sac volume, or lung densities differed among positions (controlled for recumbency order and sex). A Mauchly test was used to determine sphericity; if covariance was not found, the Greenhouse-Geisser test was used to interpret the results. Statistical software[g] was used to analyze data, and values of $P < 0.05$ were considered significant.

Results

The median age of the penguins was 13.0 years (10th to 90th percentile, 2.6 to 26.4 years; range, 0.75 to 48.0 years), and the mean weight of the penguins was 4.1 ± 0.6 kg (range, 2.8 to 5.1 kg). No complications were observed during restraint of conscious penguins or during anesthesia. Total anesthesia times ranged from 31 to 95 minutes (mean, 48.32 minutes), and all birds recovered quickly from anesthesia.

Air sac volumes were lowest in penguins in dorsal recumbency during anesthesia, compared with penguins in other positions during anesthesia and conscious penguins in ventral recumbency (**Table 1**). Significant differences were detected between male and female penguins during anesthesia regarding lung volume in dorsal recumbency ($P = 0.007$), air sac volume in right lateral recumbency ($P = 0.041$), and lung volume in right lateral recumbency ($P = 0.006$); males had higher values for all of those variables (**Table 2**). No significant differences were detected between males and females regarding air sac volume for conscious

Table 1—Air sac and lung volumes in CT images of 25 conscious Humboldt penguins (*Spheniscus humboldti*) in ventral recumbency and those same penguins anesthetized in ventral, dorsal, and right lateral recumbencies.

Variable	Mean ± SD	Range
Air sac volume (cm^3)		
Conscious ventral	$347.2 \pm 103.1^{a,b}$	187.7–550.4
Anesthetized ventral	$265.3 \pm 90.4^{a,c}$	146.5–469.0
Anesthetized dorsal	202.0* (129.2–280.3†)[b,d]	122.5–460.8
Anesthetized right lateral	$322.6 \pm 83.1^{c,d}$	185.3–498.1
Lung volume (cm^3)		
Conscious ventral	85.9 ± 18.5	55.0–127.1
Anesthetized ventral	87.1 ± 12.2	67.0–113.8
Anesthetized dorsal	80.4 ± 8.7	66.8–95.6
Anesthetized right lateral	85.8 ± 10.3	69.4–108.8

*Value reported is median. †Value reported is 10th to 90th percentile.

[a–d]Values with the same superscript letter are significantly (^aP = 0.004, $^bP < 0.001$, ^cP = 0.004, or $^dP < 0.001$) different.

Table 2—Lung and air sac volumes in CT images of male (n = 13) and female (12) anesthetized Humboldt penguins in various recumbencies.

Variable	Mean ± SD	Range
Lung volume in dorsal recumbency (cm^3)		
Males	86.6 ± 6.0	75.6–93.9
Females	75.8 ± 7.1	66.8–95.6
Air sac volume in right lateral recumbency (cm^3)		
Males	358.7 ± 71.4	284.3–475.7
Females	287.7 ± 84.5	185.3–498.1
Lung volume in right lateral recumbency (cm^3)		
Males	89.8 ± 8.7	77.3–108.8
Females	80.4 ± 7.8	69.4–97.0

penguins in ventral recumbency (P = 0.512), lung volume for conscious penguins in ventral recumbency (P = 0.301), air sac volume for anesthetized penguins in ventral recumbency (P = 0.256), lung volume for anesthetized penguins in ventral recumbency (P = 0.114), or air sac volume for anesthetized penguins in dorsal recumbency (P = 0.803). A significant difference (P = 0.023) was detected for air sac volume among positions; however, significant differences were not found for recumbency order (P = 0.167) or sex (P = 0.184). Significant differences in air sac volume were detected between conscious penguins in ventral recumbency and anesthetized penguins in ventral recumbency (P = 0.004), conscious penguins in ventral recumbency and anesthetized penguins in dorsal recumbency (P = 0.001), anesthetized penguins in ventral recumbency and anesthetized penguins in right lateral recumbency (P = 0.004), and anesthetized penguins in dorsal recumbency and anesthetized penguins in right lateral recumbency (P = 0.001). No significant (P = 0.254) difference in air sac volume was detected between conscious penguins in ventral recumbency and anesthetized penguins in right lateral recumbency. No significant difference in lung volume was detected among positions (P = 0.503) or recumbency orders (P = 0.411) or between sexes (P = 0.602).

No significant difference was detected between the right and left lung densities for conscious penguins in ventral recumbency (P = 0.972), anesthetized penguins

Table 3—Right and left lung densities in 25 conscious and anesthetized Humboldt penguins in various positions.

Variable	Median	10th to 90th percentile	Range
Right lung (g/cm^3)			
Conscious ventral	$0.508^{a,b}$	0.480–0.564	0.472–0.630
Anesthetized ventral	$0.488^{a,c,d}$	0.453–0.533	0.440–0.575
Anesthetized dorsal	$0.522^{b,c,e}$	0.489–0.553	0.485–0.610
Anesthetized right lateral	$0.509^{d,e}$	0.479–0.547	0.472–0.585
Left lung (g/cm^3)			
Conscious ventral	0.508^{f}	0.480–0.569	0.453–0.622
Anesthetized ventral	$0.482^{f,g,h}$	0.461–0.541	0.437–0.582
Anesthetized dorsal	$0.511^{g,i}$	0.491–0.556	0.472–0.632
Anesthetized right lateral	$0.492^{h,i}$	0.477–0.541	0.477–0.594

[a–i]Values with the same superscript letter are significantly ($^aP < 0.001$, ^bP = 0.052, $^cP < 0.001$, $^dP < 0.001$, ^eP = 0.011, ^fP = 0.001, $^gP < 0.001$, ^hP = 0.003, or $^iP < 0.001$) different.

in ventral recumbency (P = 0.561), or anesthetized penguins in dorsal recumbency (P = 0.201); however, there was a significant (P = 0.016) difference between right and left lung densities for anesthetized penguins in right lateral recumbency; the median value for right lungs was 0.511 g/cm^3 (10th to 90th percentile, 0.480 to 0.561 g/cm^3; range, 0.472 to 0.585 g/cm^3) and the median value for left lungs was 0.493 g/cm^3 (10th to 90th percentile, 0.477 to 0.541 g/cm^3; range, 0.477 to 0.594 g/cm^3). Because of this difference, a follow-up analysis was performed to evaluate right and left lungs separately with a general linear model. Overall, the mean and median lung densities were the highest for anesthetized penguins in dorsal recumbency and lowest for anesthetized penguins in ventral recumbency **(Table 3)**.

A significant difference was detected for right lung density among positions (P = 0.001); however, recumbency order (P = 0.477) and sex (P = 0.852) were not significant for right lung density. Significant differences in right lung density were found between conscious penguins in ventral recumbency and anesthetized penguins in ventral recumbency (P = 0.001), conscious penguins in ventral recumbency and anesthetized penguins in dorsal recumbency (P = 0.052), anesthetized penguins in ventral recumbency and anesthetized penguins in dorsal recumbency ($P < 0.001$), anesthetized penguins in ventral recumbency and anesthetized penguins in right lateral recumbency (P = 0.001), and anesthetized penguins in dorsal recumbency and anesthetized penguins in right lateral recumbency (P = 0.011; Table 3).

A significant difference was detected for left lung density among positions (P = 0.001); however, recumbency order (P = 0.664) and sex (P = 0.651) were not significant for left lung density. Significant differences in left lung density were found between conscious penguins in ventral recumbency and anesthetized penguins in ventral recumbency (P = 0.001), anesthetized penguins in ventral recumbency and anesthetized penguins in dorsal recumbency (P = 0.001), anesthetized penguins in ventral recumbency and anesthetized penguins in right lateral recumbency (P = 0.003), and anesthetized penguins in dorsal recumbency and anesthetized penguins in right lateral recumbency (P = 0.001; Table 3).

Discussion

Information in published reports regarding recommendations for recumbency during anesthesia of birds are conflicting. Some authors[23–25,29] recommend avoidance of dorsal recumbency because pectoral muscle mass is thought to compress internal organs and not allow adequate ventilation in some birds, especially species from the orders Galliformes and Anseriformes. Other authors[26–28,30] recommend the avoidance of ventral recumbency because of restriction of movement of the keel and ribs when the weight of a bird is placed on its keel. The preferred recumbency during anesthesia is likely species specific because of the wide variety of body shapes, pectoral muscle masses, and other anatomic differences among avian species. These differences are especially apparent in penguins, which are nonflighted, aquatic, and diving animals. Previously, the Humboldt penguins included in this study had typically been positioned in dorsal or right lateral recumbency during anesthesia for radiographic imaging. With increasing use of CT imaging, ventral recumbency has been used more frequently during anesthesia because of high ease of positioning. In the present study, dorsal recumbency during anesthesia led to the lowest air sac volume and the highest lung density, suggesting this position should be avoided for extended anesthesia times for this species and that ventral recumbency may be preferable.

In mammals, lungs work as both a gas exchange system and a ventilatory system. However, in birds, lungs are separate from the ventilatory system. This allows lungs in birds to be more efficient than lungs in mammals because gas exchange is performed during inspiration and expiration.[31] The air sacs do not participate in active gas exchange, acting instead as bellows; they are thin-walled and avascular.[31–36] The parabronchi and the surrounding air and blood capillaries compose the active gas exchange centers in birds.[31–32,37] The diameters of air and blood capillaries are small, compared with those of capillaries in lungs of mammals, allowing for more capillaries in an area versus lungs in mammals, leading to more efficient gas exchange.[31,38,39] This small diameter and high concentration of capillaries also prevents substantial expansion or collapse of lungs during physiologically normal conditions.[40] For this reason, lungs in birds are considered nonelastic, unlike lungs in mammals. Although significant differences in air sac and lung volumes were detected between male and female penguins for various recumbencies in the present study, males of this species are larger than females and air sac and lung volumes were larger in males, as expected. Results of analyses of data for the entire study population indicated sex had no influence on air sac or lung volumes. Recumbency and anesthesia had a negligible effect on lung volume in the penguins in this study; no significant difference was detected for lung volume in any recumbency or for conscious versus anesthetized penguins. These findings were consistent with a model of nonelastic lungs in birds, but was in contrast with results of studies performed with Pekin ducks (*Anas platyrhynchos domestica*)[16] and red-tailed hawks (*B jamaicensis*)[18]; results of those studies indicated significant lung volume changes among positions. Such differences may be species specific, and lungs with low elasticity may be beneficial for aquatic, diving birds.

In contrast to results for lung volume, significant changes were detected for lung densities among recumbencies. Changes in lung volume were especially evident for penguins in right lateral recumbency; dependent right lungs were significantly denser than nondependent left lungs. In all other positions, right and left lungs had similar densities. Therefore, we suspected that changes in lung density were attributable to fluid pooling, secondary to gravity-dependent pooling of interstitial fluid and blood in pulmonary vessels. Performance of CT after IV administration of contrast material may have allowed differentiation of density changes attributable to increased blood flow in pulmonary vasculature versus changes attributable to interstitial fluid. Increased blood pooling in the dependent lungs could contribute to ventilation-perfusion mismatch by increasing blood flow without a concomitant increase in ventilation.[41–44] Results of blood gas analyses could further allow elucidation of the effect of recumbency and lung density on respiratory gas exchange. Further physiologic studies would be needed to determine whether penguins in certain recumbencies have ventilation-perfusion mismatch.

Results indicated there were significant differences in air sac volume among positions of penguins. These changes may have been attributable to restriction of respiratory muscle movements or to shifting of coelomic organs that may have compressed air sacs in certain recumbencies. Additionally, use of anesthetics may have further depressed respiration because both inspiration and expiration require the use of respiratory musculature and anesthetics may cause muscle relaxation.[45,46] As expected, air sac volume was largest during CT of conscious penguins in ventral recumbency. During anesthesia, penguins in right lateral recumbency had the largest air sac volume. In this position, movement of the keel is not restricted as it is in ventral and dorsal recumbency. In dorsal recumbency, the weight of the keel may prevent full air sac expansion, and in ventral recumbency, the body weight on the keel may prevent full air sac expansion. Although the dependent air sacs seemed to be partially compressed by internal organs during right lateral recumbency, the nondependent air sacs inflated fully and appeared to allow the best ventilatory capacity. Computed tomography was not performed for penguins in in left lateral recumbency because of time constraints and the amount of data the scanner could process in a short time. However, air sac volume in left lateral recumbency could differ from air sac volume in right lateral recumbency because of differences in organ positioning. Penguins in dorsal recumbency had the lowest air sac volume of any position during under anesthesia; this was assumed to be attributable to compression of the air sacs by internal organs. On the basis of these findings and the lung density measurement results, we suggest that dorsal recumbency should be avoided for extended procedures in penguins because of possible hypoventilation and ventilation-perfusion mismatch. Ideally, blood gas and respiratory gas partial pressure measurements would have been compared with CT findings in this study. However, such analysis was beyond the scope of this study and further physiologic studies would be needed to determine whether volume and density measurements correlate with results of such tests.

Computed tomography was performed quickly for penguins in this study; only a single set of physiologic data, including heart rate, respiratory rate, cloacal temperature, and pulse oximetry measurements, were typically recorded during each scan. Therefore, it was not possible to determine changes in values of physiologic variables over time for penguins in each evaluated recumbency. Performance of studies that allow sufficient time for determination of changes in values over time for various recumbencies during anesthesia may allow conclusions regarding the physiologic and clinical effects of such positions. However, because air sac volume was lower in anesthetized penguins than it was in conscious penguins, it was expected that penguins would be aided by intermittent positive pressure ventilation during anesthesia because of suspected depression of the respiratory musculature secondary to anesthesia.

To prevent adverse effects attributable to anesthesia during CT, penguins were maintained in an upright position between each scan for a minimum of 3 minutes and a maximum of 5 minutes. In addition, the order in which the CT scans were performed during anesthesia was randomized to minimize sampling bias. The order in which the scans were performed had no significant effect on air sac volume, lung volume, or lung densities. Additionally, there were minimal significant differences in lung densities and lung and air sac volume attributable to sex and weight.

Computed tomography is accurate and commonly used to measure organ volume in humans.[20,47–49] Computed tomography is increasingly used to evaluate various organ systems in other animals,[22,50–52] but few studies[18,19,53] have been conducted to determine the volume and density of organs in the respiratory system. Helical CT was used in this study, which allowed procedures to be performed quickly and reproducibly for penguins in in each evaluated recumbency. In radiographs, coelomic structures often overlay lesions in air sacs, which makes identification of disease and pathological changes (eg, granulomas in the respiratory system of birds) difficult.[54] High lung density can also mask lesions in lungs in certain positions. Computed tomography allows for 3-D viewing of the respiratory system coelomic contents, making it a sensitive diagnostic tool for the early detection of lesions. As the use of CT examinations for zoological species increases, new applications may be identified. The results of the present study provided baseline information regarding air sac and lung volumes that should be beneficial for assessment of images of clinically ill birds. Changes in lung density may also occur in penguins with disease, but further research is needed to identify such changes. The use of radiation therapy treatment planning software made the volume and density measurements more precise and efficient versus other methods. In standard commercially or freely available DICOM (Digital Imaging and Communications in Medicine) viewers, these functions are commonly limited and require manual contouring of areas, which may introduce operator error. By use of the method in this study, radiodensity ranges that only incorporated lungs or air sacs allowed contouring of desired areas on all CT image slices at one time. Only minor adjustments were needed for some CT images to remove unwanted regions or to add desired regions.

The results of this study indicated that significant changes in lung density and air sac volume develop in penguins among various recumbencies during anesthesia. These changes may have substantial clinical and physiologic consequences, especially for birds in dorsal recumbency. Previous anesthetic recommendations for positioning of birds in dorsal recumbency during anesthesia may not be appropriate for penguins because of their unique body shape and anatomy. Ventral recumbency seemed to be preferable for this species and may be recommended, particularly for long procedures. Future clinical and physiologic studies are needed to determine whether lung volume and density changes detected in CT images affect cardiopulmonary system and gas exchange in Humboldt penguins and the sensitivity for detection of pathological lesions by use of CT.

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