Tracheal growth assessment in mongrel puppies (dogs) through multidetector CT

Rosa Eugenia Soriano-Rosales,¹ Beatriz Eugenia Pérez-Guillé,¹ Miguel Angel Jimenez-Bravo-Luna,¹ Susana Monroy-Santoyo,¹ Fernándo Villegas-Alvarez,¹ Arturo Carmona-Mancilla,¹ Carlos Jiménez-Gutiérrez,² Susana Leticia Elizalde-Velázquez,³ Jose Francisco Gonzalez-Zamora¹

ABSTRACT
The aim of this study was to describe the tracheal growth pattern and its zoometric relationship in related medium-sized mongrel puppies through adulthood. Fourteen puppies were studied. CT monitoring was performed monthly, starting in the 1st month of life through the 7th month and subsequently at the 9th and 12th months. Additionally, six zoometric measurements were performed. Dorsoventral (DV) and transverse (TV) diameters and the luminal area from C1 to T2 were obtained. The global tracheal growth pattern revealed an increase up to 13 times its initial size, reaching a plateau phase during the last trimester. The relationship between the DV and the TV internal diameters of the tracheal lumen did not change during growth. As previously reported, the cranial tracheal area was wider, while the caudal part gradually decreased towards T1–T2; this consideration is important since the more distal an endotracheal tube is inserted, the greater the risk that injury may occur. The linear correlation between the zoometric measurements and the tracheal ring areas was positive. This study provides evidence for the evaluation of the morphometry of the canine trachea during physiological growth using helicoidal CT as a non-invasive, accurate tool.

INTRODUCTION
In the 1980s, numerous studies in mammals described a close relationship between the dimensions of the trachea, pulmonary physiology and zoometry. In dogs, this relationship has permitted the proposal of simple prediction equations for tracheal size that consider the body mass index.¹ However, all three conditions change with growth, which suggests that the indices of the relationships between these variables change during the early stages of life.

In humans, the morphometric characteristics of the larynx and the trachea are modified according to age, gender and the measurement technique used.²–⁴ In dogs, there is little evidence that the tracheal characteristics are modified during growth in the first year of life.

The monitoring of young individuals during growth is feasible using less invasive imaging technologies.² Multislice helical CT represents a non-invasive and accurate tool for the virtual study of the airway in humans.³

The objective of this study was to describe the tracheal growth pattern in related mongrel puppies from the first month of life through adulthood and, secondarily, to correlate the tracheal growth pattern with six zoometric measurements.

MATERIALS AND METHODS
Population and sample
Fourteen related mongrel puppies from three litters were obtained from the vivarium of the National Institute of Pediatrics, Mexico City, Mexico. Six puppies were obtained from the first litter by crossing two unrelated dogs with similar phenotypic features (coat, weight and height, cranial features, neck length); the next two litters (four puppies each) were obtained by the inbreeding of father-daughter and mother-son, respectively. The health condition of the animals was assessed three times per week during the study, paying special attention to symptoms that could suggest a respiratory disease (fever, runny nose, pink eye with discharge, tachypnoea, dyspnoea, cyanosis, stridor, abdominal breathing, lethargy); other parameters evaluated were weight and height gain, normal or abnormal feeding and vital signs.

CT monitoring was performed monthly, starting with the 1st month of life through the 7th month and subsequently at the 9th and 12th months, at ±10 days. During the same period, six zoometric measurements were performed, including weight (kg), thoracic perimeter at the level of xyphoid process (cm), neck length from the jaw bone to sterno-
the neck length (cm), scapular-ileal length from superior scapular spinae to iliac spine (cm) and scapular-carpal length from scapular spinae to metacarpophalangeal joint (cm).

**Tomographic measurements**

CT measurements were performed in a Siemens 4 AG Medical Solutions CT Scan (Forchheim, Germany). A 30 mAs low-dose localisation scan was performed from the base of the skull to the hemidiaphragms. The standardised technical parameters were as follows: 80 kV, 100 mAs, 10 mm×1 mm cut thickness and 75 per cent reconstruction increment (equivalent to 0.75 mm). To reconstruct the images, a field of vision with 50 cm was used, together with a filter for mediastinum, which included a medium-smooth kernel, a window centre of 100 and a window level of 30.

Each dog was placed in the supine position on the tomographic table. The dog’s head was fixed with pads to maintain the same position in all evaluations (Fig 1).

The images were measured and evaluated with the workstation Somatom Sensation 4 Siemens software.

We obtained the maximum dorsoventral (DV) and transverse (TV) diameters and the internal area (A) from C1 to T2; all measurements were taken at the mid-corpus level of the corresponding vertebral body. The tracheal length from C1 to T6 was also measured, taking the cricoid cartilage as the upper limit and the carina as the lower limit.

A certified radiologist performed the measurements, which were taken twice with an intraobserver coefficient variation of 92 per cent. The measurement technique was performed hands-free with a region of interest (ROI) tool, delineating the trachea in its internal contour and measuring the DV and TV diameters with the linear measuring tool at the point of the maximum diameters.

**Anaesthetic management**

The tomographic procedures were performed under fixed anaesthesia for a short duration. No endotracheal tube was used to avoid modifying the shape or form of the trachea. The anaesthesia included a mixture of 7 mg/kg of xylazine, 0.05 mg/kg of atropine and 10 mg/kg of tiletamine/zolazepam administered intramuscularly.

**Ethical considerations**

The animals were treated according to the Official Standard NOM-062-1999 following the internal guidelines of the vivarium of the INP and in accordance with the Guide for the Care and Use of Laboratory Animals.

**Statistical analysis**

The results of the measurements obtained from the tomographic images and zoometric measurements are shown as the mean with a CI of 95 per cent. Normality was determined by Shapiro Wilks test. To evaluate the tracheal growth, Pearson’s correlation coefficient and simple linear regression were performed. For the comparison between area, DV and TV diameters of the different tracheal rings along the period of time a multivariate analysis of variance (MANOVA) test within the general linear model for repetitive measures analysis was done.

Pearson’s correlation coefficient was used for the analysis of the type of relationship among the following variables: zoometric measurements, tracheal length and area of the tracheal rings. The level of assumed significance for all analyses was <0.05. The analysis was performed using the SPSS V.19 (IBM Corporation, New York, EUA).
RESULTS
Six females and eight males were included in this study. The initial and final averages of the zoometric measurements are as follows: weight (W) $2.0$ kg (1.5–2.4) vs 18.3 kg (16.2–20.4), thoracic perimeter (TP) 29.4 cm (24.8–33.9) vs 60.7 cm (58.4–62.9), neck length (NL) 18.8 cm (16.2–21.3) vs 39 cm (37.4–40.5), neck perimeter (NP) 20.8 cm (17.5–24) vs 37.7 cm (35.9–39.6), scapular-ileal length (SIL) 18.2 cm (13.2–23.2) vs 57.0 cm (54.3–59.6) and scapular-carpal length (SCL) 16.9 cm (14.9–18.8) vs 40.4 cm (38.2–42.6).

The structural characteristics of the cervical trachea at the beginning and end of the research are shown in Fig 2a–f. All of the tracheal cartilages could be identified by the tenth month. Before the ninth month, only a tubular structure without rings was observed. In 7/14 dogs, the relationship of the first cervical vertebra with the first tracheal ring and the sixth thoracic vertebra with the carina suffered caudal displacement during the growth of the dog, thus these vertebrae were not included in the analysis.

The growth pattern of the area of the tracheal rings is shown in Table 1, demonstrating an increase up to

![FIG 3: CT scan multiplanar slices of the trachea. (a) Axial slice showing the dorsoventral (corresponding to DV) and transverse (TV) tracheal diameters at the level of thoracic vertebra. (b) Coronal slice. (c) Sagittal slice.](image-url)
Differences between the means and the age in months were noticed (Greenhouse-Geisser test F=168.411, P=0.000) as well as a linear trend between the means (F=328.093, P=0.000). Shaded areas show the highest and lowest values of each month.

13 times its initial value (C7). This pattern exhibited quadratic character in all the different tracheal rings, with a decrease in the growth rate from the second semester of life observed in the graphics. The most representative rings are shown in Fig 3. Regarding the tracheal length, the average size was 11.1±1.1 cm (95% CI 10 to 12.2) after the first measurements, reaching 24.7±3.2 cm (95% CI 22.8 to 26.6) by the end of the study (Fig 3).

A difference in the luminal area of the cervical rings (C2 vs C7) was identified in the first measurement; however, from the second month to the end of the study, the main differences were observed between C2 and T1 (Table 1); the first being the larger and the latter being the smallest in all of the measurements. It is noteworthy that after T1, all of the thoracic rings tended to widen. The result of the ratio between the DV diameter and TV diameters of the tracheal rings was close to 1 at almost all levels and in all measurements (Tables 2 and 3).

However, at the T1 level, the ratio was 1.02 (95% CI 0.8 to 1.4), while at the C2 level, since the DV diameter was larger, the result was 1.25 (95% CI 0.94 to 1.38). The tracheal shape is distinct in its cephalic portion because the ring at the C2 level adopts a subtle vertical elliptical shape, whereas the rest of the trachea tends to be circular.

The linear correlation between the zoometric measurements and the tracheal ring areas was positive in all cases, as shown in Table 4. The highest correlation was observed in the cephalic portion of the tracheal rings as well as in the tracheal length.

**DISCUSSION**

In the group of puppies analysed, the global tracheal growth pattern revealed an increase of up to 13 times its initial size, reaching a plateau phase during the last trimester. The widest ring area was exhibited at the cervical region. We observed that the tracheal rings became progressively denser and that the tomographic characteristics of the developed trachea were evident by the ninth month (Fig 2). Regardless of the growth of the
ring areas over the course of the entire study, the relationship between the internal measurements (DV/TV diameter) of the trachea did not change during growth.

There was a positive correlation between the increase in the zoometric measurements and the area of the tracheal rings, primarily at the proximal portion of the trachea and between the zoometric measurements and the tracheal length.

Based on the classic description of the canine trachea, the structure extends from the middle of the first cervical body to the fifth thoracic vertebra, where it bifurcates into the right and left bronchi immediately dorsal to the body to the fifth thoracic vertebra, where it bifurcates.

### TABLE 3: Transverse (TV) diameter growth pattern

| Age in months | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Diameter cm: mean, 95% CI, median | 0.92 | 0.88 | 0.84 | 0.80 | 0.76 | 0.72 | 0.68 | 0.64 | 0.60 | 0.56 | 0.52 | 0.48 |

### TABLE 4: Correlation between zoometric measurements and luminal area/tracheal length

| Zoometric measurements | C2  | C3  | C4  | C5  | C6  | C7  | T1  | T2  | T3  | T4  | T5  | T6  |
|------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Peso weight            | 0.845* | 0.838* | 0.834* | 0.822* | 0.80* | 0.738* | 0.746* | 0.749* | 0.762* | 0.776* | 0.718* | 0.875* |
| Thoracic perimeter     | 0.847* | 0.811* | 0.804* | 0.796* | 0.778* | 0.717* | 0.723* | 0.724* | 0.734* | 0.743* | 0.695* | 0.880* |
| Neck length            | 0.958* | 0.812* | 0.811* | 0.797* | 0.786* | 0.716* | 0.736* | 0.744* | 0.757* | 0.761* | 0.697* | 0.863* |
| Neck perimeter         | 0.792* | 0.769* | 0.766* | 0.746* | 0.726* | 0.656* | 0.667* | 0.685* | 0.703* | 0.712* | 0.645* | 0.825* |
| Scapular-ileal length  | 0.862* | 0.815* | 0.819* | 0.809* | 0.784* | 0.722* | 0.733* | 0.743* | 0.764* | 0.774* | 0.720* | 0.898* |
| Scapular-carpal        | 0.862* | 0.790* | 0.790* | 0.774* | 0.760* | 0.697* | 0.705* | 0.710* | 0.715* | 0.710* | 0.657* | 0.848* |

*P<0.05.
base of the heart. Thus, the trachea has a cervical portion and a thoracic portion. The ventral and lateral walls are composed of hyaline cartilage arches. Observations in this study differed regarding the anatomical relationship between the trachea and the vertebral bodies because it was inconstant during growth. In the first measurement, in 7/14 animals, the trachea started at the level of C2, while in late measurements, caudal displacement between the rings and the vertebrae was observed.

The trachea in adults is described as cylindrical and irregular, in which the most dilated area is the initial part (first cervical vertebra) and a narrowing occurs at the thoracic inlet. This situation is consistent with our observations, where the initial area is wider (second cervical vertebra) and the distal part gradually decreases towards T1–T2 (Fig 4). This consideration is important since the more distal an endotracheal tube is inserted, the greater pressure will be that is exerted on the inner walls of the trachea with the cuff, carrying more risk of endotracheal injury.

As described by Kara and others in adult dogs, we observed no significant difference between the DV and TV diameters of the tracheal lumen in puppies during normal development. This finding suggests that this relationship is preserved through life. In contrast to our study, observed a modest increase in the TV diameter, where the DV diameter was greater. This difference could be explained by the position in which the patients were lying during CT, which was supine in our study versus prone in Kara’s study, thus encouraging tracheal hyperextension.

Concerning the growth rate of the ring area and the tracheal length, a plateau phase was observed during the last trimester, which correlated with the zoometric measurements. The relationship between growth and weight was described in cross-sectional studies from the 1970s. This study corroborated that this relationship was maintained throughout the development of the puppies.

The linear correlation demonstrated in this study between the values obtained at the C2 level during the growth of the puppy supported the usefulness of the equation proposed by Avki and others for the calculation of the size of the endotracheal tube in medium-sized breed puppies. However, extrapolating these results to other breeds of dogs is a limitation due to the great phenotypic variation in dogs.

The use of non-invasive technologies for the study of the trachea, tomographic studies are useful for describing structural alterations. In humans, helical tomography is ideal for the exploration of the trachea because it permits making three-dimensional reconstructions similar to virtual bronchoscopy. Since 1997, three trials have described the morphometric characteristics of the trachea of the adult dog using CT. performed cross-sectional studies of the trachea in healthy dogs.
in 2001 and 2004 and concluded that this method was an easy but expensive way to safely perform permitted repeated measurements over time. In the analysis of the multidetector CT (MDCT) images that we produced, we corroborated the advantages of this tool. Due to the speed of the study, the anaesthesia administered to the puppies was shallow and short.

This study provides evidence for the evaluation of the morphometry of the trachea during the growth of individuals using helicoidal CT as a non-invasive and accurate tool. Additionally, normal values can be described when using this tool in a group of medium-sized breed dogs.

A limitation of this study is the variation of growth rates at which dogs, of different breeds, sizes and gender go through at each stage of development.

CONCLUSIONS

The global tracheal growth pattern revealed an increase of up to 13 times its initial size, reaching a plateau phase during the last trimester. Regardless of the accelerated tracheal growth during the first year of life, its anatomy did not change over time. Zoometric measurements could be used to determine the tracheal area, irrespective of the age of the dog.

Acknowledgements The authors are grateful to the animal technicians and staff of the Institute’s (INP) Vivarium and Imagenology Department, especially Sandra Zaragoza Huerta.

Contributors RES-R: leadership, planning, animal handling, measurement and follow up, data review. BEP-G: planning, animal handling, measurement and follow up, data review. MAJ-B-L: planning, animal handling, measurement and follow up. SM-S: planning, review materials, analysis of data. FV-A: tomographic measurements, image reconstruction and analysis. JFG-Z: planning, review materials, data transfer and analysis.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Ethics approval This study was approved by the Research Committees and the Institutional Animal Care and Use Committee (Comité Institucional para el Cuidado y Uso de Animales de Laboratorio) of the National Institute of Pediatrics (Instituto Nacional de Pediatría) (INP: 37/2006).

REFERENCES

1. Avki S, Yigitstarlan K, Ozgel O. Comparison of airway pathophysiology with some phenotypic parameters in dalmatian puppies: a practical method to estimate endotracheal tube size. *Vet Anaesth Analg* 2006;33:24–7.
2. Kara ME, Turan E, Dabanoglu I, et al. Computed tomographic assessment of the trachea in the German shepherd dog. *Ann Anat* 2004;186:317–21.
3. Kamel KS, Lau G, Stringer MD. In vivo and in vitro morphometry of the human trachea. *Clin Anat* 2009;22:571–9.
4. Monnier P. Applied surgical anatomy of the larynx and trachea. In: *Pediatric airway surgery*. Springer-Verlag, 2010:7–31.
5. Griscom NT, Wohl ME. Dimensions of the growing trachea related to age and gender. *AJR Am J Roentgenol* 1986;146:233–7.
6. Institute for Laboratory Animals Research. *Guide for the care and use of laboratory animals*. 8th edn: The National Academies Press, 2011.
7. Animal Welfare Act and Animal Welfare Regulations. *United states department of agriculture*. US: CFR. Animal Welfare Regulations, 2013. 9 § 3.129.
8. Evans HE, Christensen GC. *Miller’s Anatomy of the Dog*. W.B. Saunders, 1979.
9. Dallman MJ, Brown EM. Statistical analysis of selected tracheal measurements in normal dogs and dogs with collapsed trachea. *Am J Vet Res* 1984;45:1033–7.
10. Dabanoglu I, Ocal MK, Kara ME. A quantitative study on the trachea of the dog. *Anat Histol Embryol* 2001;30:57–9.
11. ten Hallers EJ, Raikhorst G, Marres HA, et al. Animal models for tracheal research. *Biomaterials* 2004;25:1533–43.
12. López-Herranz GP. Endotracheal intubation: importance of the cuff pressure over the tracheal epithelium. *Revista Medica del Hospital General de Mexico*, 2013;76: 153–61.
13. Karra ME, Karaman ZC, Dabanoglu I, et al. Computed tomographic measurements of the trachea in dogs. *Dtsch Tierarztl Wochenschr* 2001;108:164–7.
14. Brown RH, Mitzner W. Understanding airway pathophysiology with computed tomography. *J Appl Physiol* 2003;95:854–62.
15. Fike JR, Druy EM, Zook BC, et al. Canine anatomy as assessed by computerized tomography. *Am J Vet Res* 1980;41:1823–32.