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Tidal Breathing Flow-Volume Loop Analysis for the Diagnosis and Staging of Tracheal Collapse in Dogs

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Background: Tracheoscopy is generally used for the diagnosis of tracheal collapse (TC) in dogs; yet, it is costly, requires anesthesia, and can irritate the airway. The tidal breathing flow-volume loop (TBFVL) is a safe, quick, and noninvasive pulmonary function test currently used in humans.

Hypothesis: TBFVL will differentiate dogs with TC from healthy controls and contribute to disease grading.

Animals: Twenty-eight dogs with naturally occurring TC and 10 healthy controls.

Methods: Cross-sectional, prospective clinical study: The 38 dogs were assigned to one of 4 groups based on tracheoscopy results: group A (n = 10, healthy controls), group B (n = 10, grade I TC), group C (n = 10, grade II TC), and group D (n = 8, grade III TC). The TBFVL measurement was performed on all dogs and loops were assessed for their shape. Forty-four TBFVL parameters were calculated.

Results: Two types of TBFVL shapes were identified: Type I, representative of the 10 healthy controls, and Type II, representative of the 28 dogs with TC. Statistical analysis showed the dogs could be differentiated into healthy or affected by TC by 3 indices, TE/TI (expiratory time divided by inspiratory time), TI/TTOT (inspiratory time divided by total respiratory time), and EF75/IF75 (expiratory flow at end tidal volume plus 75% end tidal volume divided by inspiratory flow at end tidal volume plus 75% end tidal volume). The TC could also be graded as mild-moderate (grades I and II) or severe (grade III), showing a diagnostic value of 97.4%.

Conclusion and Clinical Importance: TBFVL is accurate, quick, noninvasive, and safe and can contribute to the diagnosis of TC in dogs.

Key words: Animals; Canine; Pulmonary function tests; Tracheal disease.

Pulmonary function tests are widely used in human medicine to contribute to the diagnosis and assess treatment for respiratory diseases.[1] One of the most common tests is the acquisition of a maximum expiratory and inspiratory flow-volume (MEFV, MIFV) curve, which demands patient cooperation and, unfortunately, cannot be routinely used in animals.[1,2] However, over the past 20 years the tidal breathing flow-volume loop (TBFVL), a modified type of MEFV and MIFV originally applied in infants, has been successfully used for the evaluation of respiratory diseases in dogs and cats.[3–9]

Tracheal collapse (TC) is a common disorder in middle aged, toy, and miniature breed dogs. It is considered a structural abnormality of the trachea and is characterized by a variable degree of tracheal ring dorsoventral flattening, accompanied by flaccidity of the dorsal tracheal membrane.[10,11] Compatible history, clinical examination, and imaging (radiography, fluoroscopy, ultrasound) may aid the diagnosis, but they all have limitations.[11] The gold standard for diagnosing and determining the stage of the disease is tracheoscopy.[11–17] Unfortunately, the requirement for anesthesia and airway irritation from the endoscope are major disadvantages. In animals already decompensated, tracheoscopy may lead to further airway irritation, increased respiratory effort, and difficult recovery.[17] Therefore, there is a need for noninvasive diagnostic methods.

The TBFVL is a quick, simple, noninvasive, and well-tolerated diagnostic method with minimum patient risk. To our knowledge, there has been only one study that used TBFVL for the evaluation of dogs with TC.[5] The

Abbreviations:

AUC	areas under the curve
EF	expiratory flow rate
FEF50%	flow rate at 50% of forced expiratory volume
FIF50%	flow rate at 50% of forced inspiratory volume
IF	inspiratory flow rate
MEFV	maximum expiratory flow-volume
MIFV	maximum inspiratory flow-volume
PEF	peak expiratory flow rate
PIF	peak inspiratory flow rate
T.EXP.AUC	total area under the expiratory curve
T.INSP.AUC	total area under the inspiratory curve
TBEV	tidal breathing expiratory volume
TBFVL	tidal breathing flow-volume loop
TC	tracheal collapse
TE	expiratory time
TI	inspiratory time
TM	tracheomalacia
TTOT	total respiratory time
VT	tidal volume

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number of cases in this study was small, the diagnosis of TC had not been confirmed endoscopically, and the dogs had concurrent chronic bronchitis and/or laryngeal paresis. Yet, this study set the foundation for use of TBFVL in dogs with TC. Information gathered from literature review was helpful and inspiring, but did not reveal any specific TBFVL parameters that could be increased or decreased in dogs with TC.[3,5,8] It could be assumed that since TC is characterized by reduction of tracheal lumen diameter, TBFVL parameters reflecting flow, such as peak and midtidal expiratory and inspiratory flow rates (PEF, PIF, EF_{50}, IF_{50}), as well as expiratory and inspiratory flow rates at end tidal volume plus 75% end tidal volume (EF_{75}, IF_{75}) were expected to be decreased.[1–6,8] In flow-limiting respiratory diseases, time (expiratory time [TE], inspiratory time [TI]) related parameters are expected to be increased. This hypothesis is based in the assumption that decreased flow rates probably lead to increased breathing time, which eventually would permit the dog to breath a sufficient air volume.[1–6] Flow and time related parameters were the primary variables of interest in this study. In accordance to a study in healthy and bronchitic cats a greater number of TBFVL parameters were checked in an attempt to determine whether other parameters are influenced from TC.[8]

Materials and Methods

Study Design

A cross-sectional prospective clinical study was designed to test 2 hypotheses: (1) TBFVL is a valuable diagnostic method in differentiating dogs affected by TC from healthy controls, and (2) TBFVL can contribute to staging canine TC.

Animals

Thirty-eight client-owned dogs admitted to our clinic, during the period from January 2005 to May 2007, were enrolled in the study. Twenty-eight of them were affected by naturally occurring TC (grades I–III) and 10 were normal controls. Endoscopic evaluation was performed in all the dogs in order to confirm and determine the grade of TC, where it existed, according to the staging system established by Tangner and Hobson.[12] Tracheoscopies were performed using a flexible fiberoptic bronchoscope.[a] Dogs were allocated into 1 of 4 groups: group A (n = 10), consisted of 10 healthy dogs; group B of 10 dogs affected by grade I TC; group C of 10 dogs affected by grade II TC; and group D of 8 dogs affected by grade III TC.

Inclusion Criteria

This study was approved by the Faculty of Veterinary Medicine, ethics committee. The care and use of animals complied with local animal welfare laws, guidelines, and policies. All dogs included in the study met the same inclusion criteria: (a) they belonged to a small or toy breed, (b) their age ranged from 1 to 16 years, (c) they were serologically and parasitologically negative for *Dirofilaria immitis*, (d) fecal examination with Baermann and flotation and sedimentation methods for lung worms was negative, (e) if they were diagnosed with congestive heart failure, it was not more than stage Ia–Ib according to the grading system of the International Small Animal Cardiac Health Council,[18] (f) they had never injured or had a surgery performed to the respiratory system, (g) they were not affected by any other disease, (h) they had no other respiratory disease, and (i) they had not received any medication that could have influenced respiratory function for at least 7 days before admission.

Diagnostic Evaluation

Detailed history was followed by physical examination, CBC, serum biochemistry and urinalysis, serology and parasitology for *Dirofilaria immitis*, fecal examination, and ECG.[b,c] If a cardiac murmur was detected, cardiac function status was further evaluated by echocardiography.[d] Arterial blood gas analysis was performed in 22 of the 38 dogs with a bed-side analyzer; the samples were handled in accordance with the manufacturer's instructions.[e]

Lateral inspiratory and expiratory radiographs of the neck and thorax as well as a inspiratory dorsoventral radiograph of the thorax were obtained.[f] Dogs were premedicated with acepromazine (0.05 mg/kg IM) in combination with butorphanol (0.2 mg/kg IM).[g,h] Anesthesia was induced and maintained by IV administration of propofol.[i]

During endoscopy, the function and anatomical integrity of the larynx and tracheobronchial tree were evaluated. TC was graded and the location of the collapsed tracheal segment, as well as its length, were determined and measured by marking the endoscope. Endoscopy was always performed by the same endoscopist who was not aware of the results of TBFVL analysis.

The TBFVL measurement was performed on all dogs included in the study. The TBFVL measurement was always scheduled 1 day before radiographical and endoscopical evaluation, to avoid the influence of general anesthesia and irritation of the respiratory tract from the endoscope. In addition, it was always performed while the dogs were awake, calm, unsedated, and in a standing position in the same quiet, nonstressful environment. Care was taken not to compress the neck, thorax, or abdomen of the dog. Also, the head was kept in a normal position with the mandibles at a 45°–90° angle with the neck. No attempts were made to train any of the study dogs to the procedure. For TBFVL measurements, each dog wore a tight-fitting facemask of appropriate size, which included the angles of the lips.[j] Before the beginning of the procedure, the dogs were kept by their owners and wore the mask for 2–3 minutes to get used to it. The facemask was attached to a pressure sensor,[k] connected to a high sensitivity transducer[l] that transformed pressure signals into electrical signals (0–5 V). The analog signals were digitized with a data acquisition card[m] and analyzed with specially designed software.[n] The software displayed flow-volume loops and flow versus time curves during the procedure. The system was calibrated before each measurement with a standard volume syringe.[o] For each dog, TBFVL measurements were recorded for approximately 10 minutes and data of 8–10 representative breaths were obtained. Criteria for the selection of loops included lack of artifacts (eg, movements), a difference of $\leq$5% between inspiratory and expiratory volumes, and a respiratory frequency of $\leq$60 breaths per minute.[5]

Loops were initially assessed for their shape. Subsequently, tidal volume (VT), tidal breathing expiratory volume at 0.1 and 0.5 seconds after the beginning of expiration (TBEV 0.1, TBEV 0.5), respiratory rate, PEF, PIF, EF_{50}, IF_{50}, EF, and IF at end tidal volume plus 12.5, 25, and 75% end tidal volume ($EF_{12.5}$, $IF_{12.5}$, EF_{25}, IF_{25}, EF_{75}, IF_{75}), as well as their ratios were calculated. TE, TI, and total respiratory time and their ratios were determined. Finally, the areas under the curve (AUC) at peak, 50 and 25% of expiratory and inspiratory flows (AUC PEF, AUC PIF, AUC 50% EF, AUC 50% IF, AUC 25% EF, AUC 25% IF), as well as the total area under the expiratory and inspiratory curve (T.EXP.AUC, T.INSP.AUC), and selected ratios of these parameters were calculated, as proposed for cats and humans (Table 1).[7,8]

Table 1. Mean and standard deviation ($\bar{X} \pm$ SD) or geometric means and 95% confidence intervals[a] of the tidal breathing flow volume loop parameters from 10 healthy dogs (group A), 20 dogs with grades I and II tracheal collapse (group BC), and 8 dogs with grade III tracheal collapse (group D) (2nd classification).

a/a	Parameters of TBFVL	Abbreviation	Group A (n = 10)	Group BC (n = 20)	Group D (n = 8)	P^{**}	CV (%)
Inspiratory variables							
1	Peak inspiratory flow	PIF	0.19 ± 0.03 b*	0.18 ± 0.02 b	0.06 ± 0.03 b	.08	8.1
2	Inspiratory flow at end tidal volume plus 75% end tidal volume	IF75	0.15 ± 0.02 a	0.11 ± 0.01 ab	0.06 ± 0.02 b	.009	9.0
3	Inspiratory flow at midtidal volume	IF50	0.15 ± 0.02 a	0.1 ± 0.01 ab	0.05 ± 0.02 b	.006	7.9
4	Inspiratory flow at end tidal volume plus 25% end tidal volume	IF25	0.11 ± 0.02 a	0.09 ± 0.01 ab	0.05 ± 0.02 b	.02	9.9
5	Inspiratory flow at end tidal volume plus 12.5% end tidal volume	IF12.5	0.09 ± 0.01 b	0.07 ± 0.01 b	0.04 ± 0.01 b	.04	18.2
6	Peak inspiratory flow divided by inspira tory flow at midtidal volume	PIF/IF50	1.13 ± 0.02 b	1.13 ± 0.01 b	1.12 ± 0.02 b	.95	5.9
7	Peak inspiratory flow divided by inspira tory flow at end tidal volume plus 25% end tidal volume	PIF/IF25	1.38 ± 0.06 b	1.33 ± 0.03 b	1.27 ± 0.06 b	.41	8.6
8	Peak inspiratory flow divided by inspira tory flow at end tidal volume plus 12.5% end tidal volume	PIF/IF12.5	1.97 ± 0.13 b	1.59 ± 0.08 b	1.47 ± 0.14 b	.04	16.7
9	Inspiratory flow at midtidal volume divided by inspiratory flow at end tidal volume plus 25% end tidal volume	IF50/IF25	1.23 ± 0.03 b	1.17 ± 0.02 b	1.13 ± 0.03 b	.11	6.9
10	Inspiratory flow at end tidal volume plus 25% end tidal volume divided by inspiratory flow at end tidal volume plus 12.5% end tidal volume	IF25/IF12.5	1.41 ± 0.06 a	1.2 ± 0.04 b	1.17 ± 0.06 b	.02	9.0
Expiratory variables							
11	Peak expiratory flow	PEF	0.2 ± 0.03 b	0.2 ± 0.02 b	0.21 ± 0.03 b	.95	9.2
12	Expiratory flow at end tidal volume plus 75% end tidal volume	EF75	0.2 ± 0.03 b	0.19 ± 0.02 b	0.19 ± 0.03 b	.96	10.0
13	Expiratory flow at midtidal volume	EF50	0.18 ± 0.03 b	0.17 ± 0.02 b	0.18 ± 0.03 b	.87	11.3
14	Expiratory flow at end tidal volume plus 25% end tidal volume	EF25	0.12 ± 0.02 b	0.11 ± 0.01 b	0.12 ± 0.02 b	.85	15.8
15	Expiratory flow at end tidal volume plus 12.5% end tidal volume	EF12.5	0.09 ± 0.02 b	0.07 ± 0.01 b	0.07 ± 0.02 b	.67	24.4
16	Peak expiratory flow divided by expira tory flow at midtidal volume	PEF/EF50	1.2 ± 0.03 b	1.18 ± 0.02 b	1.13 ± 0.03 b	.33	6.4
17	Peak expiratory flow divided by expira tory flow at end tidal volume plus 25% end tidal volume	PEF/ EF25	1.72 ± 0.07 b	1.84 ± 0.05 b	1.74 ± 0.08 b	.25	14.7
18	Peak expiratory flow divided by expira tory flow at end tidal volume plus 12.5% end tidal volume	PEF/EF12.5	2.87 ± 0.26 b	3.03 ± 0.16 b	3.11 ± 0.27 b	.82	22.4
19	Expiratory flow at midtidal volume divided by expiratory flow at end tidal volume plus 25% end tidal volume	EF50/EF25	1.59 ± 0.09 b	1.54 ± 0.06 b	1.52 ± 0.09 b	.88	10.0
20	Expiratory flow at end tidal volume plus 25% end tidal volume divided by expiratory flow at end tidal volume plus 12.5% end tidal volume	EF25/EF12.5	1.66 ± 0.11 b	1.6 ± 0.07 b	1.83 ± 0.11 b	.22	13.8
Ratios of expiratory to inspiratory variables							
21	Peak expiratory flow divided by peak inspiratory flow	PEF/PIF	1.37 ± 0.12 b	1.74 ± 0.08 b	3.35 ± 0.13 a	< .0001	10.8
22	Expiratory flow at end tidal volume plus 75% end tidal volume divided by inspiratory flow at end tidal volume plus 75% end tidal volume	EF75/IF75	1.36 ± 0.1 c	1.77 ± 0.07 b	3.31 ± 0.11 a	< .0001	10.7
23	Expiratory flow at midtidal volume divided by inspiratory flow at midtidal volume	EF50/IF50	1.36 ± 0.14 b	1.65 ± 0.09 b	3.36 ± 0.15 a	< .0001	12.3

Table 1. (Continued).

a/a	Parameters of TBFVL	Abbreviation	Group A (n = 10)	Group BC (n = 20)	Group D (n = 8)	P^{**}	CV (%)
24	Expiratory flow at end tidal volume plus 25% end tidal volume divided by inspiratory flow at end tidal volume plus 25% end tidal volume	EF25/IF25	1.13 ± 0.1 b	1.27 ± 0.06 b	2.54 ± 0.1 a	< .0001	17.1
25	Expiratory flow at end tidal volume plus 12.5% end tidal volume divided by inspiratory flow at end tidal volume plus 12.5% end tidal volume	EF12.5/IF12.5	0.94 ± 0.10 b	0.98 ± 0.06 b	1.71 ± 0.11 a	< .0001	25.7
Time variables							
26	Inspiratory time in seconds	TI	1.14 ± 0.14 b	1.3 ± 0.09 b	1.3 ± 0.09 b	.64	12.7
27	Expiratory time in seconds	TE	1.21 ± 0.11 a	1.06 ± 0.07 ab	0.76 ± 0.12 b	.03	13.2
28	Expiratory time divided by inspiratory time	TE/TI	1.11 ± 0.04 a	0.83 ± 0.03 b	0.62 ± 0.05 c	< .0001	13.3
29	Inspiratory time divided by total respiratory time	TI/TTOL	0.48 ± 0.01 c	0.55 ± 0.01 b	0.62 ± 0.02 a	< .0001	6.6
Global variables							
30	Tidal volume	VT	91.58 ± 15.43 b	100.73 ± 9.73 b	69.36 ± 16.05 b	.24	7.5
31	Tidal volume divided by inspiratory time	VT/TI	88.39 ± 15.34 b	86.15 ± 9.67 b	54.13 ± 15.95 b	.21	16.4
32	Respiratory rate	R R	26.83 ± 2.48 b	28.34 ± 1.56 b	23.2 ± 2.58 b	.23	11.0
33	Tidal expiratory volume at 0.1 seconds after the beginning of the expiratory phase	TBEV 0.1	4.93 ± 0.86 a	1.4 ± 0.54 b	2.52 ±0.89 ab	.007	10.5
34	Tidal expiratory volume at 0.5 seconds after the beginning of expiration	TBEV 0.5	29.71 ± 5.1 a	11.85 ± 3.19 b	14.95 ± 5.26 ab	0.02	9.8
35	Area under the expiratory curve from peak expiratory flow to end tidal volume divided by area under the inspiratory curve from the peak inspiratory flow to the beginning of the breath	AUC PEF/ AUC PIF	1.24 ± 0.26 b	1.47 ± 0.17 b	3.07 ± 0.27 a	< .0001	19.3
36	Area under total expiratory curve divided by area under total inspiratory curve	T.EXP.AUC/ T.INSP.AUC	1.21 ± 0.11 b	1.5 ± 0.07 b	2.89 ± 0.11 a	< .0001	11.5
37[a]	Area under the inspiratory curve from the peak inspiratory flow to the beginning of the breath	AUC PIF	3978 a (1,921, 8,238)	2966 a (1,773, 4,963)	738 b (317, 1,775)	.39	18.9
38[a]	Area under the inspiratory curve from 50% of peak inspiratory flow to the beginning of the breath	AUC 50% PIF	2332 b (1,156, 4,703)	1716 b (1,045, 2,818)	522 b (238, 1,144)	.40	15.3
39[a]	Area under the inspiratory curve from 25% of peak inspiratory flow to the beginning of the breath	AUC 25% PIF	987 b (479, 2,035)	715 b (429, 1,192)	218 b (97, 489)	.41	19.2
40[a]	Area under total inspiratory curve	T.INSP.AUC	5204 b (2,618, 10,344)	3835 b (2,359, 6,234)	1143 b (530, 2464)	.40	12.9
41[a]	Area under the expiratory curve from peak expiratory flow to end tidal volume	AUC PEF	3988 b (2,000, 7,953)	3938 b (2,417, 6,416)	2177 b (1,006, 4,710)	.63	17.8
42[a]	Area under the expiratory curve from 50% of peak expiratory flow to end tidal volume	AUC 50% PEF	2042 b (1,005, 4,150)	2284 b (1,383, 3,771)	1420 b (643, 3,138)	.72	16.2
43[a]	Area under the expiratory curve from 25% of peak expiratory flow to end tidal volume	AUC 25% PEF	713 b (345, 1,475)	739 b (442, 1,235)	415 b (184, 935)	.64	22.8
44[a]	Area under total expiratory curve	T.EXP.AUC	5458 b (2,713, 10,980)	5845 b (3,566, 9,582)	3553 b (1626, 7,762)	.73	14.0

CV, coefficient of variation.

*Means in the same row followed by different letters (a, b, c) differ significantly according to the Bonferroni test.

**P, significance of the differences between the 3 groups according to analysis of covariance results.

Statistical Analysis

For the comparisons of TBFVL parameters among the 4 groups of dogs, analysis of covariance, with 1 between-factor (group of dogs with 4 levels) and 1 covariate (age), was used. Age was selected as a covariate, because it has negative impact upon the functional capacity of the respiratory system.[19] All the values were determined from the 6 representative loops of each dog and were calculated as the mean absolute value (±SD), which were used to establish the mean value (±SD) for each of the 4 groups. For the comparisons of means, the Bonferroni test was used. Before the analysis, the values of the parameters No 37–44 were transformed according to the $\log_{10}$ transformation, in order to achieve homogeneity of variances. In the table, the geometric mean and 95% confidence interval are provided.[20] Cluster analysis was applied to discriminate the ability of TBFVL parameters to predict the group in which a dog should be classified, without taking into account the primary endoscopical classification.[21,22] For detection of the parameters with the highest diagnostic value (the capability of a parameter to predict the degree of a disease), the stepwise discriminant analysis method was applied.[23] For all statistical testing, the experiment's Type I error rate was set to 0.05. All analyses were performed by SPSS ver. 11.5.[p]

Results

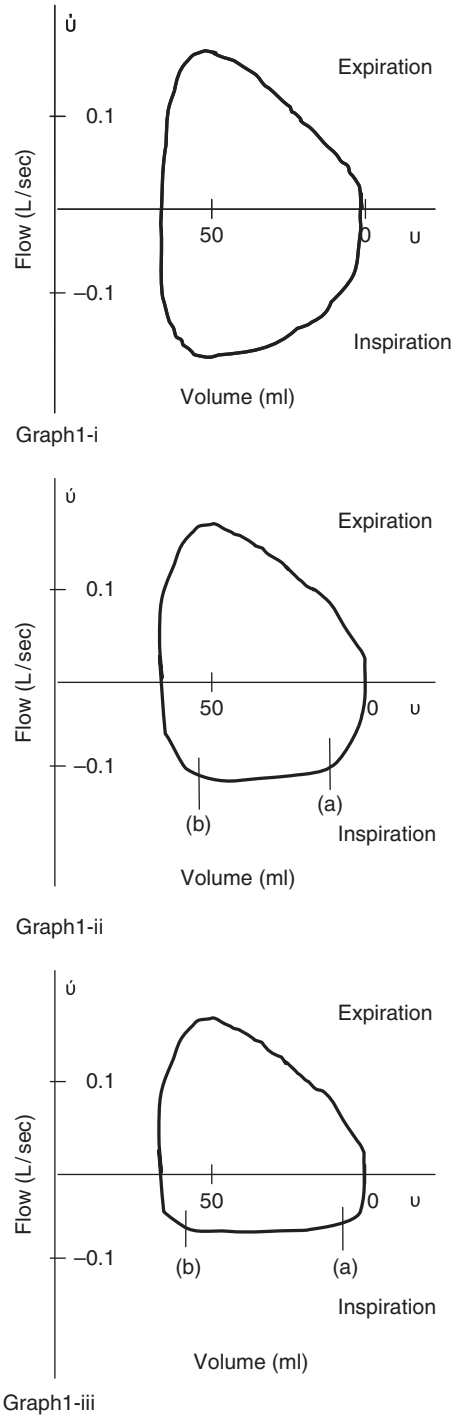
Of the 38 dogs enrolled in the study, 19 (50%) were intact males and the rest females (11 of 19 intact). The dogs belonged to various breeds, including 10 (26%) Yorkshire Terriers, 8 (21%) Miniature Poodles, 7 (18%) cross-bred Miniature Poodles, 7 (18%) miniature Mongrels, 4 (11%) Miniature Pinschers, and 1 (3%) each Pomeranian and Spitz. The ages of all dogs ranged from 1 to 14 years (median, 8 years) and their body weight from 1.8 to 13.5 kg (median, 4.4 kg). The ages of the dogs diagnosed with TC ranged from 3.5 to 14 years (median, 9 years) and their body weight from 1.8 to 13.5 kg (median, 3.9 kg).

Cough was the main clinical complaint and physical examination finding (28 of 28 dogs with TC, 100%). Dental disease was present in 26 (68%) dogs. Heart murmur of the mitral valve was detected in 7 (18%) dogs, but history, signalment, ECG, thoracic radiographs, and echocardiography classified their condition as stage Ia cardiac failure. The arterial blood gas analysis results were within normal limits in the dogs subjected to this test (22 of 38, 58%).

In the vast majority of the dogs diagnosed with TC, the trachea was collapsed at the cervical portion (22 of 28, 79%), whereas in the remaining 6 dogs, the trachea was collapsed throughout its entire length. Upon endoscopy, the length of the collapsed portion was measured and ranged from 2 to 11 cm (median, 3.9 cm) in group B, from 3 to 10 cm (median, 5.9 cm) in group C, and from 2 to 10 cm (median, 5.4) in group D dogs.

Among the dogs with TC, 20 (71%) had a radiographic appearance compatible with tracheal luminal attenuation. The radiographic appearance of 1 group A dog was falsely positive for TC, while the trachea appeared normal in 6 group B and 2 group C dogs. Radiographs of all group D dogs were suggestive of TC.

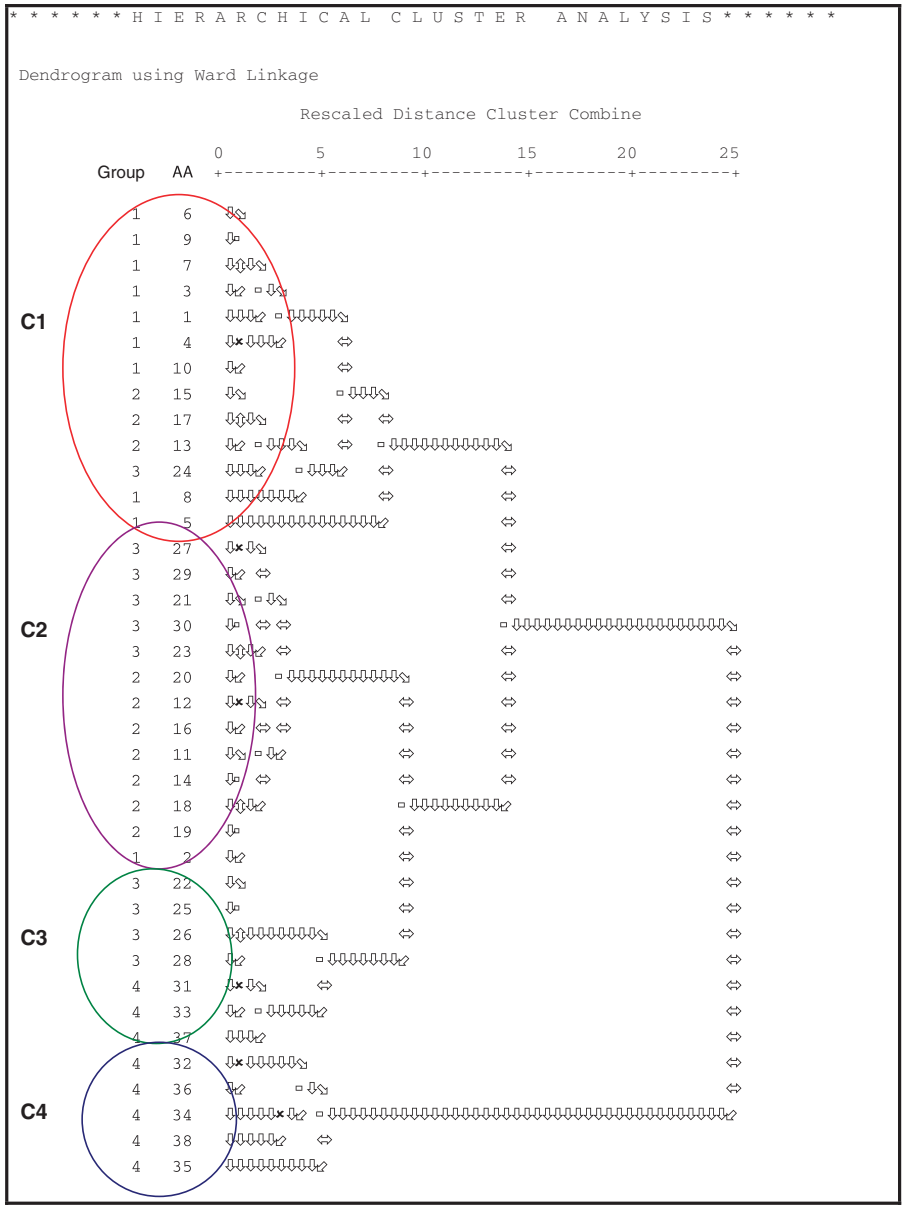
Two types of TBFVL shapes were identified in the dogs of the present study. Type I (Graph 1-i), was seen in the 10 group A dogs (healthy controls) and was similar to that described previously for healthy dogs.[5] Type II TBFVL (Graph 1-ii and Graph 1-iii representative of dogs affected by stage I/II or III TC, respectively) was



Graph 1. Two types of tidal breathing flow-volume loop shape in the 38 dogs. (i) Tidal breathing flow-volume loop shape of 10 healthy dogs (type I). Both parts (inspiratory-expiratory) are of the same size. (ii) and (iii) Tidal breathing flow-volume loop shape of 28 dogs with stages I/II and III, respectively, of tracheal collapse (type II). The 2 parts of the loop are not of the same size. The inspiratory part is flattened. (a) Beginning of inspiratory plateau, (b) end of inspiratory plateau.

encountered for the remaining 28 dogs, belonging to groups B, C, and D (dogs with TC) and was indicative of a nonfixed (dynamic), upper airway obstruction. Specifically, type II showed an inspiratory plateau at its middle part, while the inspiratory area seemed to be smaller.

Thirteen parameters were found to differ significantly among the dogs of the 4 groups, but their diagnostic values varied. Statistically significant increases were noticed in AUCPEF/AUCPIF (No. 35), T.EXP.AUC/T.INPS.AUC (No. 36), PEF/PIF (No. 21), EF50/IF50 (No. 23), EF25/IF25 (No. 24), EF12.5/IF12.5 (No. 25), TI/TTOL (No. 29) and EF75/IF75 (No. 22); while TE/TI (No. 28), IF50 (No. 3), IF25 (No. 4), IF75 (No. 2), and TBEV 0.1 (No. 33) decreased significantly in the dogs with TC compared with the healthy controls. In an attempt to evaluate the ability of the 13 parameters, as a diagnostic total, to detect the correct group to which a given dog belongs to without taking into account the primary endoscopical classification, it was revealed that group B dogs could not be easily distinguished from group C. Moreover, 3 dogs with stage I TC and 1 with stage II were allocated into group A dogs (Dendrogram 1). It was concluded that the ability of these 13 parameters, to distinguish the stage of TC in a dog, was limited and we decided to merge groups B and



Dendrogram 1. Representation of 4 clumps of healthy and with various stages of tracheal collapse dogs that had risen from hierarchical cluster analysis for which the 13 parameters of tidal breathing flow-volume loop were taken into account as a diagnostic total. Also, the allocation of each dog into the 4 clumps may be seen. C1, clump 1; C2, clump 2; C3, clump 3; C4, clump 4; 1, group A; 2, group B; 3, group C; 4, group D.

C into 1 group (group BC). This group consisted of dogs with grades I and II TC. The 2nd statistical analysis showed that 16 TBFVL parameters, with variable diagnostic values, differed significantly among the 3 groups of dogs. Statistically significant increases were noticed in AUCPEF/AUCPIF (No. 35), T.EXP.AUC/T.INP.AUC (No. 36), PEF/PIF (No. 21), EF50/IF50 (No. 23), EF25/IF25 (No. 24), EF12.5/IF12.5 (No. 25), TI/TTOL (No. 29) and EF75/IF75 (No. 22); while decrease was noted in IF25/IF12.5 (No. 10), TE (No. 27), TE/TI (No. 28), IF50 (No. 3), IF25 (No. 4), IF75 (No. 2), TBEV 0.1 (No. 33), and TBEV 0.5 (No. 34) between the dogs with TC compared with the healthy controls (numbers indicate the row of Table). These 16 parameters showed a substantial ability to detect the exact group of a given dog, without taking into account the primary endoscopical classification. Most of the dogs affected by TC were allocated accurately into their groups (BC and D) (Dendrogram 2). Unfortunately, the same 4 dogs with TC that were allocated as healthy dogs, with the former classification, were classified as healthy. The next step was to detect the parameters with the highest diagnostic capability. From both classifications the same 3 parameters, TE/TI, TI/TTOL, and EF75/IF75, were selected but, according to the discriminant analysis validation procedure, they showed different diagnostic values. In the 2nd classification (groups A, BC, and D) they showed a diagnostic value of 97.4%, while in the 1st (groups A, B, C, and D) 94.7%. To distinguish the stage of TC in a dog, 2 equations must by calculated, which take into account the 3 parameters as a total:

$$F1 = -21.685 + 4.037 \times (TE/TI) + 22.639 \times (TI/TTOL) + 2.906 \times (EF75/IF75)$$

$$F2 = -35.220 + 16.868 \times (TE/TI) + 32.842 \times (TI/TTOL) + 1.348 \times (EF75/IF75)$$

If the values of TE/TI, TI/TTOL, and EF75/IF75 of a dog are known, then the dog can be allocated into 1 of the 3 groups simply by solving the 2 equations and projecting the results on the territorial map of Graph 2.

Discussion

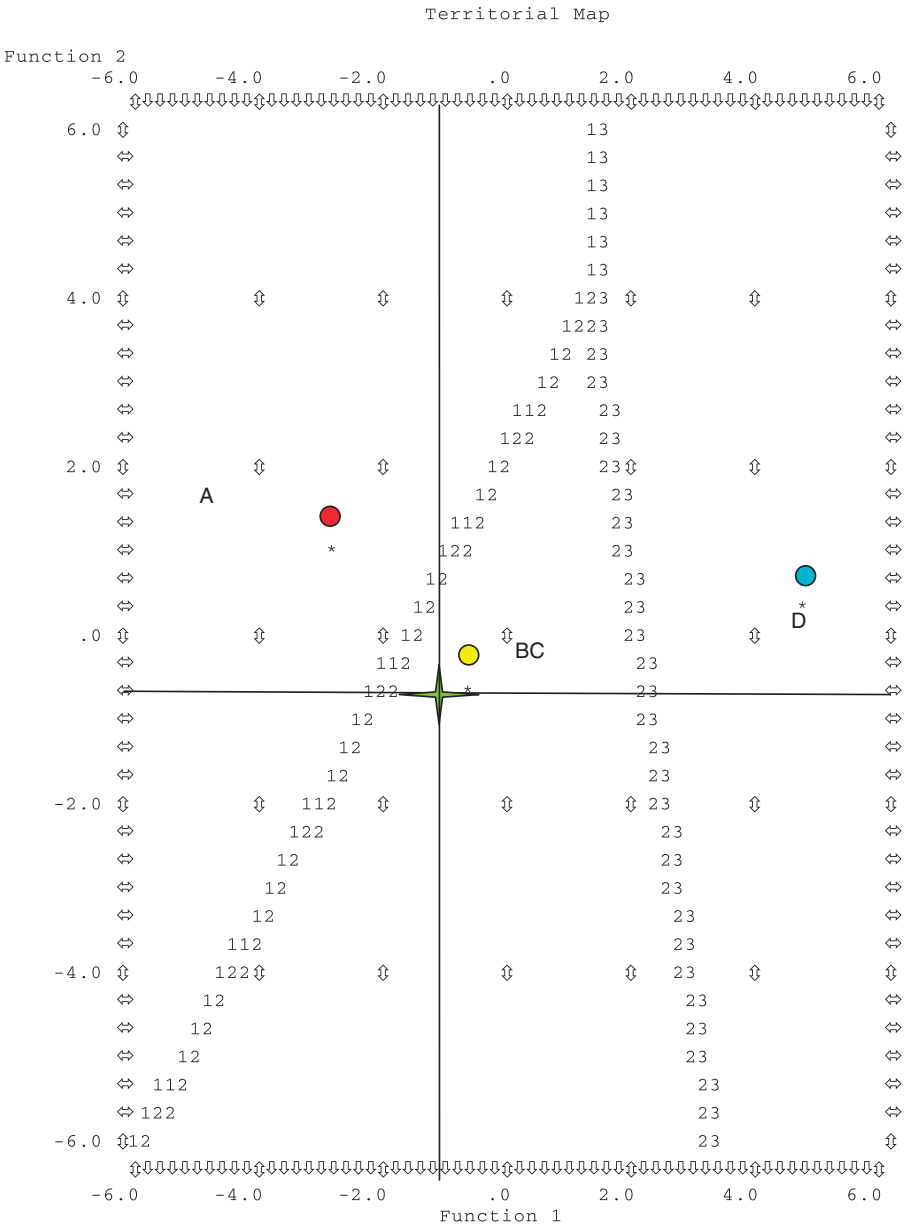
In this study, the epidemiological data of the 28 dogs with TC were similar to those mentioned for dogs with TC. Overrepresentation has been noticed in Yorkshire Terriers (39–83.3%),[13,24] followed by Miniature Poodles.[13] The higher percentage of mixed breed dogs in our study may be because of the increased population of these dogs in Greece. No sex predisposition was found, in accordance with the results of other studies[13,15,17] and, in contrast, to the male predisposition documented in humans with tracheomalacia (TM).[25] Finally, the median age (9 years) and median body weight (3.9 kg) of dogs with TC were similar to those reported previously.[26]

Dental disease was apparent in most of the dogs (68%) with TC, supporting the findings of Macready et al.[26] A possible correlation between bacterial tracheal infection and dental disease, which exacerbates the clinical signs of these dogs, must be considered.[27] One fifth of the dogs with TC also presented with a heart murmur because of mitral valve regurgitation. This is a common finding (10–30%) in canine TC,[13,24,26,27] perhaps reflecting that both diseases share the same breed predisposition.[24,26] Arterial blood gas analysis did not reveal any abnormalities, most likely because none of the dogs exhibited respiratory distress upon examination.

In our study, most of the dogs (22 of 28, 79%) with TC presented with collapse of the cervical portion of the trachea. Collapse was noticed in the entire length of the trachea in the remaining dogs. None of the dogs showed collapse solely of the intrathoracic portion of the trachea, confirming the findings of other studies.[13,14,16,28] It is worth mentioning that when the stage of TC deteriorated, the trachea length increased, affecting the intrathoracic portion. In another study, TC was noticed in the entire length of the trachea in most of the dogs (79.2%) with grades III and IV TC.[24]

In humans and animals, the 1st in the evaluation of TBFVL is careful inspection of its shape.[3,4,5,6,29–31] In our study, 2 highly reproducible types of TBFVL shapes were identified (Graph 1a, 1b). The first was compatible with a normal loop shape.[5] The second showed a dynamic upper airway obstruction, similar to that described by McKiernan and Johnson[32] and Rozanski and Hoffman.[33] TBFVL could be a valuable method for the initial evaluation of a dog suspected to be affected by TC. In humans, the sensitivity of TBFVL for detecting laryngomalacia and TM is almost 100 and 97.4%, respectively.[31] Because our study did not include dogs simultaneously affected by TC and a concurrent disease (eg, laryngeal paralysis), specificity and sensitivity cannot be estimated. In humans with TM, the disturbance of loop shape is seen in the expiration, which is probably because of the intrathoracic location of the larger part of the trachea.[25] In the dogs of our study that were affected by both cervical and intrathoracic TC, the shape of the loop showed a plateau just in the inspiration, similar to dogs that had just cervical TC. This result may reflect the different ratio of the cervical to the intrathoracic part of the trachea in dogs[34] compared with humans[25] and strengthens the conviction that the trachea in dogs is mostly in the upper respiratory system.[34]

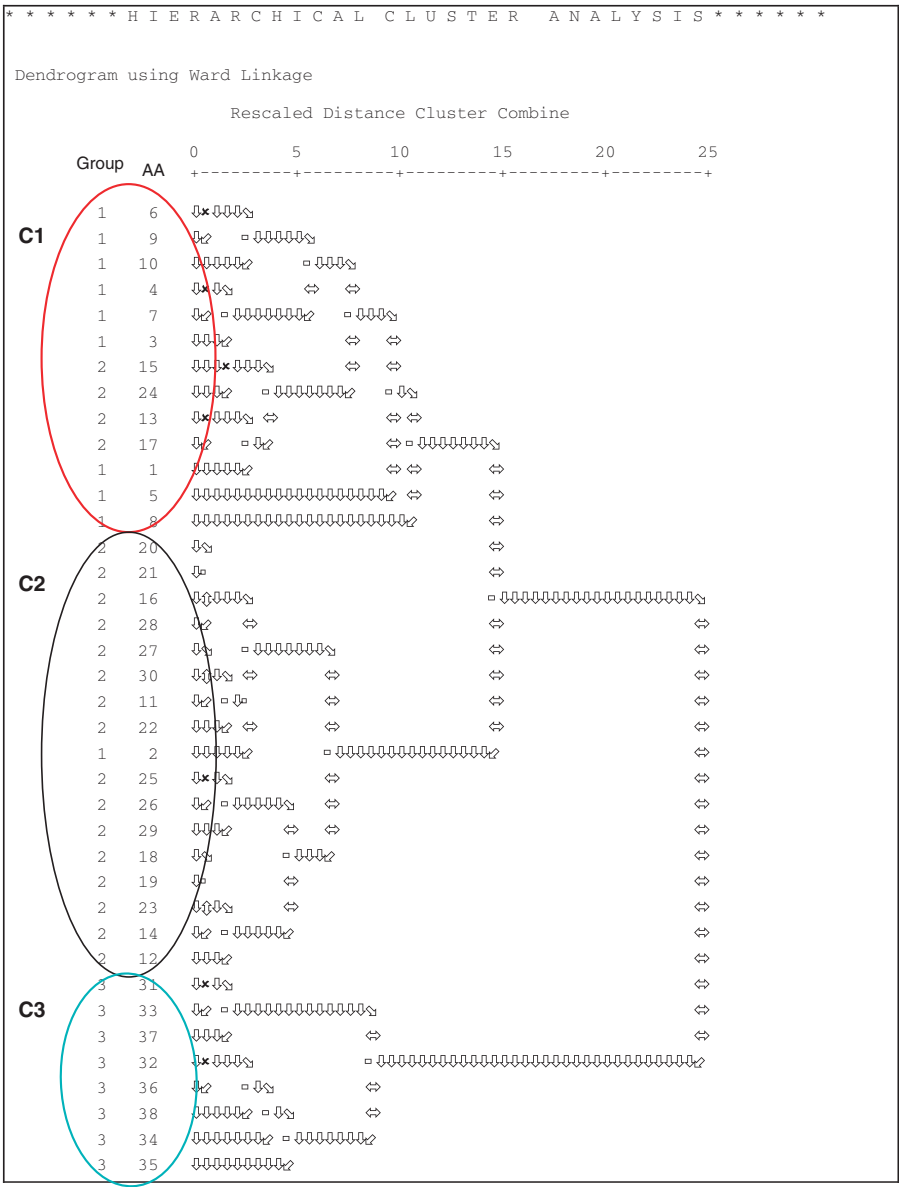
Dogs allocated into grades I and II TC, based on TBFVL, were not consistent with allocations following tracheoscopy. Although the latter is the gold standard in diagnosing and staging TC, its main limitation is the inability to evaluate the degree of dorsal tracheal membrane attenuation objectively.[35] Tracheoscopy is not accurate in diagnosing TM in humans, although appropriate breathing maneuvers by the patient improve its results. However, the mild/moderate and severe forms of TM are recognized.[36] When the dynamic nature of TC[27] is taken into account, the mild (grade I) and the moderate (grade II) forms of the disease may not be easily differentiated, even by an experienced endoscopist.[26] According to the results of our study, it seems TBFVL could also recognize the dynamic form of TC. It should be emphasized that its diagnostic value was improved when groups B and C were merged into one.



Graph 2. Territorial map of the 3 groups of healthy and with various stages of tracheal collapse dogs (A, BC, and D).
Example: Dog no. 19; If the values of TI, TE, TTOL, EF75, and IF75 are known, the 2 equations can be calculated and
F1 = − 21.685 + 4.037 × (0.8325) + 22.639 × (0.5456) + 2.906 × (1.6124) = − 1.2867
F2 = − 35.220 + 16.868 × (0.8325) + 32.842 × (0.5456) + 1.348 × (1.6124) = − 1.085
According to the results the location + of the dog into the territorial map may be seen.
1, group A (10 healthy dogs); 2, group BC (20 dogs with grades I and II TC); 3, group D (8 dogs with grade III TC).
Center of Group A.
Center of group BC.
Center of group D.

In both classifications, 3 dogs with grade I and 1 dog with grade II TC were allocated with healthy dogs based on TBFVL (Dendrograms 1, 2). However, after merging groups B and C into one, these dogs were closer to group BC (Dendrogram 2). Based on the history, clinical examination findings, and TBFVL shape of these 4 dogs, findings were compatible with TC. Flow and volume are variably influenced by effort, breathing duration, pleural space pressure, airway resistance, etc.[2] Consequently, it is not always feasible to evaluate flow and volume by only 1 diagnostic method. In humans, TBFVL was not able to establish the diagnosis of TM in 2.6% of patients.[31,37]

In dogs with TC, the inspiratory plateau started just after the beginning (point a, Graph 1-ii) and terminated just before the end of inspiration (point b, Graph 1-ii). The parameters IF25, IF50, and IF75 decreased signifi-



Dendrogram 2. Representation of 3 clumps of healthy and with various stages of tracheal collapse dogs that had risen from hierarchical cluster analysis for which the 16 parameters of tidal breathing flow-volume loop were taken into account as a diagnostic total. Also, the allocation of each dog into the 3 clumps may be seen. C1, clump 1; C2, clump 2; C3, clump 3; 1, group A; 2, group BC; 3, group D.

cantly (Table 1) and were representative of the flow rates in 3 different lung volumes. In human infants and young children with TM, the inspiratory curve also flattened at middle and high inspiratory lung volumes.[37] Amis and Kurpershoek[5] had also found that IF25 and IF50 decreased in dogs with TC also affected by chronic bronchitis and laryngeal paresis. The decrease in inspiratory flows was also emphasized by the statistical significant increase of EF25/IF25, EF50/IF50, and EF75/IF75. In humans, FIF50% (flow rate at 50% of forced inspiratory volume) and FEF50%/FIF50% (flow rate at 50% of forced expiratory volume divided by flow rate at 50% of forced inspiratory volume) are the 2 most representative parameters for the diagnosis of dynamic upper airway obstruction.[38]

Resistance is inversely proportional to the radius of the tracheal lumen diameter taken to the 4th power.[1,15] Increased resistance is overcome by either increased effort or decreased air flow. Increased effort presents clinically as respiratory distress, while reduced flow presents as an increase in inspiratory time. This is documented by the equations Flow = Volume/Time and Resistance = Pressure/Flow.[1,39] Since most of the dogs in the present study did not present with respiratory distress, the compensating mechanism was reduction of flow. In humans with upper airway obstructions, it was suspected that increased effort was associated with an unpleasant sensation contributing to reduced flow rates.[40]

Despite the gradual reduction in PIF, no statistically significant difference could be found between groups B,

C, and D. A similar observation was reported in dogs with laryngeal paralysis.[4] However, the statistically significant increase in PEF/PIF emphasized the reduction of PIF. The use of indices that are expressed as ratios of absolute values is very useful because they have the ability to "neutralize" the influence of subject dependent variables such as body weight, breed, or fluctuations in breathing effort.[4,5] Ratios in adults and infants[2] have uncovered differences that would have not been found by calculating just absolute values.

The parameters that are associated with the calculation of the area under the flow-volume curve have been used as an objective measure of bronchodilator efficacy in humans[7] and for the diagnosis of feline chronic bronchial disease.[8] These parameters are considered to be very sensitive indicators and they allow even mild deviations from normal to be tracked.[7,9] Moreover, the AUC is mostly influenced by changes in flow rate and not from VT values.[9] Therefore, we calculated these parameters in dogs with TC. We found the statistically significant increase in T.EXP.AUC/T.INSP.AUC and AUCPEF/AUCPIF also emphasized the substantial decrease of inspiratory flow in these dogs.

Two of the most valuable parameters, for the diagnosis and staging of TC, were found to be TE/TI and TI/TTOL, which respectively decreased and increased significantly. This may have been because of an increase in TI, since VT remained unchanged and flow was reduced in dogs with TC. This may present clinically as a prolonged inspiratory phase.

The statistically significant and gradual reduction in TBEV 0.1 and TBEV 0.5, as the stage of TC worsened, was an unexpected finding. Based on this finding, expiratory flow should have been reduced or PEF should have moved to the right of the expiratory loop, reflecting its later onset in the expiratory phase. Since neither occurred, TBEV 0.1 and TBEV 0.5 reduction may have been associated with a transient and quick attenuation of expiratory volume at the beginning of expiration. This was probably because of invagination of the dorsal tracheal membrane into the tracheal lumen,[12,36] which contributed to a small increase in resistance and a reduction in the very initial VT.

Eventually, 3 (TE/TI, TI/TTOL, and EF75/IF75) of the 16 parameters showed substantial diagnostic value and the equations were formed (F1, F2). The calculation of these equations and the projection of their results to the territorial map could lead to 97.4% accuracy in the diagnosis and staging of TC in dogs.

Because of the inclusion criteria of this study (dogs that enrolled would have been affected exclusively by TC and not from concurrent diseases), the number of dogs enrolled was limited. Further studies having the ability to randomly sample dogs from larger numbers of dogs in the groups may increase the accuracy of these results.

In conclusion, TBFVL is an accurate, quick (10–20 minutes), and noninvasive method for the diagnosis and staging of TC in dogs. The expense for the equipment was approximately 15,000 euros. Only 3 parameters (TE/TI, TI/TTOL, and EF75/IF75) are necessary to calculate a definitive diagnosis and stage of the disease with a diagnostic value of 97.4%, without affecting the dog's clinical condition. Although further studies are necessary to ensure the accuracy of TBFVL in dogs that are affected simultaneously by other air flow limiting conditions, our results support that TBFVL can be used as diagnostic tool for routine evaluation of dogs at risk for TC. Moreover, although it was not in the scope of this study, it must be mentioned that TBFVL analysis might be used to assess response of the patient to, medical or surgical, therapy, and to monitor disease progression.

Footnotes

[a] Karl Storz 60003VB, Karl Storz GmbH Co, Tuttlingen, Germany
[b] Snap Canine Heartworm PF, IDEXX, Westbrook, ME
[c] SIMPLISCRIPTOR EK 100, Hellige, Freiburg, Germany
[d] Apogee, Advanced Technologies Laboratory, Bothell, WA
[e] i-STAT, SDI, Abbott Laboratories, Waukesha, IL
[f] Polydoros 80, Siemens, Munich, Germany
[g] Acetylpromazine, Acepromazine; Agroseed Candilidis, Athens, Greece
[h] Butorfanol, Butomidor; Richter-Pharma, Wels, Austria
[i] Propofol, Propofol Abbott 1%; Abbott Laboratories, Karachi, Pakistan
[j] Small animal anaesthesia mask, Eickemeyer, Tuttlingen, Germany
[k] D-lite, Datex Ohmeda, Louisville, KY
[l] Spirotransducer, Infoproject, Thessaloniki, Greece
[m] PCL-711B, PC-Multilab Card, sample rate 100/seconds; Advantech Europe, Eindhoven, the Netherlands
[n] Spirometer, Infoproject
[o] Model 1000 CalSTAT, Volume Calibration Standard, Electronics Inc, MED, St Louis, MO
[p] SPSS for Windows ver. 11.5, Chicago, IL

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