

# Use of volumetric capnography to identify pulmonary dysfunction in horses with and without clinically apparent recurrent airway obstruction

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**Objective**—To investigate whether volumetric capnography indices could be used to differentiate between horses without recurrent airway obstruction (RAO) and horses with RAO that were in clinical remission or that had clinically apparent RAO.

**Animals**—70 adult Swiss Warmblood horses (20 used for pleasure riding and 50 used for dressage or show jumping).

**Procedure**—Horses were allocated to 4 groups on the basis of history, clinical signs, results of endoscopy, and cytologic findings (group 1, 21 healthy horses; group 2, 22 horses with RAO that were in remission; group 3, 16 horses with mild RAO; group 4, 11 horses with exacerbated RAO). Expiratory volume and CO<sub>2</sub> curves were recorded by use of a computerized ultrasonic spirometer. Volumetric capnograms were plotted, and derived indices were calculated.

**Results**—Dead-space volume (V<sub>d</sub>) was calculated by use of the Bohr equation (V<sub>DBohr</sub>) and for physiologic V<sub>D</sub> (V<sub>Dphys</sub>). Ratios for V<sub>DBohr</sub> to expiratory tidal volume (V<sub>T</sub>) and V<sub>Dphys</sub> to V<sub>T</sub> as well as an index of effective CO<sub>2</sub> elimination were significantly different among groups of horses. Age and use of the horses also significantly affected volumetric capnography indices.

**Conclusions and Clinical Relevance**—Ratios of V<sub>DBohr</sub> to V<sub>T</sub> and V<sub>Dphys</sub> to V<sub>T</sub> as well as an index of effective CO<sub>2</sub> elimination were sufficiently sensitive measures to distinguish between healthy horses and horses with RAO in remission. To optimize the ability of volumetric capnography indices to differentiate among horses in heterogeneous populations, it is important to account for effects of age and specific use of the horses. (*Am J Vet Res* 2003;64:338–345)

Obstruction of the bronchioles and alveoli in horses has been commonly measured by use of a pneumotachograph-esophageal balloon method, which allows measurements of maximal changes in transpul-

monary or pleural pressure, dynamic lung compliance, and pulmonary resistance.<sup>1</sup> Measurement of Pao<sub>2</sub> and difference in PAO<sub>2</sub> and PaO<sub>2</sub> (AaDO<sub>2</sub>) have also been widely used to assess pulmonary gas exchange, especially in clinical practice.<sup>2</sup> Shortcomings of these established pulmonary function tests are their invasiveness and lack of sensitivity. These measures only detect airway obstruction once it has become relatively severe and is clinically apparent.<sup>3</sup> Airway obstruction is clinically apparent at rest in horses with recurrent airway obstruction (RAO) that is exacerbated (ie, crisis that typically is evident within 24 hours after exposure to hay), but it is not clinically apparent in horses with RAO that is in remission or in horses with mild RAO.<sup>4</sup> However, these milder forms of airway disease in horses are clinically important. Therefore, more sensitive pulmonary function tests have been developed, including forced oscillation combined with histamine bronchoprovocation,<sup>5</sup> forced expiration,<sup>3</sup> and volumetric capnography.<sup>6,7</sup>

A graph of expired CO<sub>2</sub> concentration versus expired volume is termed the volumetric capnogram or single-breath diagram for CO<sub>2</sub> (SBD-CO<sub>2</sub>). Volumetric capnography provides indices of the existence of nonuniformities in the distribution of pulmonary blood flow relative to ventilation (ie, alveolar ventilation/perfusion [ $\dot{V}_A/\dot{Q}$ ] mismatching).<sup>8</sup> As an effort-independent method of pulmonary function testing, volumetric capnograms may be recorded in awake,<sup>6,7,9,10</sup> anesthetized, or mechanically ventilated horses.<sup>8</sup> A number of pulmonary function indices can be obtained from the volumetric capnogram in a noninvasive manner (eg, anatomic dead-space fraction or dead-space fraction determined by use of the Bohr equation). Calculation of physiologic and alveolar dead-space fractions necessitates collection of arterial blood samples. Volumetric capnography indices have high repeatability<sup>6,7</sup> and correlate with the severity of clinical signs in horses with varying degrees of bronchiolar or alveolar disease.<sup>9</sup>

We hypothesized that pulmonary function testing based on volumetric capnography indices would allow detection of differences in  $\dot{V}_A/\dot{Q}$  mismatching between healthy control horses without evidence of airway disease, horses with RAO that were in remission, horses with mild RAO, and horses with RAO that was exacerbated. Furthermore, correlations between volumetric capnography indices and arterial blood gas tensions, end-tidal CO<sub>2</sub> concentrations, and alveolar CO<sub>2</sub> fractions were investigated.

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## Materials and Methods

**Animals**—Seventy Swiss Warmblood horses were included in the study (20 were used for pleasure riding, and 50 were sport horses used for dressage or show jumping). Forty-three horses were part of a group of 250 horses owned by the Swiss National Horse Center. The remaining 27 horses had been referred to the equine clinic of the University of Berne for clinical examination of the respiratory tract.

All 70 horses were hospitalized for 3 days at the equine clinic of the University of Berne. Horses were housed in stalls bedded with straw and fed hay and a grain mixture, except for a few horses that were housed in stalls bedded with wood shavings and fed a substitute for hay; these horses were housed and fed in accordance with conditions similar to those at their home farm.

**Clinical examination and auxiliary tests**—Pulmonary health of each horse was evaluated during clinical examination and by use of simple auxiliary diagnostic methods, including arterial blood gas analyses, tracheobronchoscopy, and cytologic examination of tracheobronchial aspirates. All horses were exercised for 15 minutes (5 minutes each of walking, trotting, and cantering) to detect any coughing during or after exercise as well as prolonged recovery (increased respiratory rate) after exercise. Potential confounding influences of viral and bacterial infections were monitored by use of hematologic assessments (CBC counts with differential cell counts) and measurements of body temperature.

The skin over the distal third of the right jugular vein was aseptically prepared and infiltrated with an anesthetic.<sup>b</sup> Then, a 20-gauge needle was inserted into the right external carotid artery. The needle was inserted immediately before volumetric capnography was initiated with the horse wearing a facemask and the flow head attached. The facemask met criteria of the American Thoracic Society in that it did not affect respiration (ie, dead-space volume of the face mask was < 6% of expiratory tidal volume [ $V_T$ ]), and flow resistance was undetectable during tidal breathing at rest and was 6 millibars at a flow rate of 100 L/s). Three consecutive arterial blood samples were obtained for blood gas measurements. Each sample was collected during a period of 3 breaths with the horses breathing quietly as soon as possible after the recording was started. During the measurements, a thermometer<sup>c</sup> was inserted into the rectum of each horse and was clipped to the tail; rectal temperature was used to adjust blood gas and capnography measurements. Arterial blood gas samples were stored on ice and immediately analyzed at the end of the experiment by use of a blood gas analyzer.<sup>d</sup> The blood gas analyzer was automatically calibrated to an accuracy of  $\pm 0.5\%$  relative to  $O_2$  and  $CO_2$  reference gases, and adjustment on the basis of the rectal temperature of each horse was performed by the analyzer in accordance with the operator's manual.<sup>d</sup>

Values for  $AaDO_2$  were calculated as described by Capro et al<sup>11</sup> by use of the following equation:

$$AaDO_2 = \left( (0.2093 \times [PB - PH_2O]) - (Paco_2 \times [0.2093 + \{1.0 - 0.2093/R\}]) \right) - PaO_2$$

where PB was measured atmospheric pressure,  $PH_2O$  was water pressure adjusted on the basis of rectal temperature,  $PaO_2$  and  $Paco_2$  were mean values obtained for the 3 arterial samples, and R was the expiratory exchange ratio (assumed to be 0.8).<sup>2</sup> Atmospheric pressure at time of arterial blood gas analysis ranged from 705 to 714 mm Hg (mean  $\pm$  SD,  $711 \pm 3.5$  mm Hg) measured with a certified mercury barometer<sup>c</sup> that was corrected on the basis of temperature, altitude, and latitude.

**Classification of horses**—Horses were categorized into 4 groups on the basis of history and results of clinical and auxiliary examinations. Group 1 comprised 21 control horses (8 mares, 11 geldings, and 2 stallions) that ranged from 4 to 12 years of age (mean  $\pm$  SD,  $8.6 \pm 3$  years). These horses did not have a history of respiratory tract disorders despite living in an environment in which they were constantly exposed to hay and straw. Abnormalities were not detected during clinical examinations including an exercise test, arterial blood gas analysis ( $PaO_2 > 90$  mm Hg,  $AaDO_2 < 7$  mm Hg), tracheobronchoscopy (isolated small spots of mucus; liquid to moderately viscous mucus), and cytologic examination of tracheobronchial aspirates (< 10% polymorphonuclear neutrophils in tracheal secretions). Group 2 comprised 22 horses (3 mares, 17 geldings, and 2 stallions; 4 to 15 years old [mean,  $9.5 \pm 4.2$  years]) that had RAO but were in remission. These horses had a history of RAO with clinically apparent increased respiratory effort at rest (dyspnea) when fed hay, and they were bedded with wood shavings and fed grass silage. Abnormalities were not detected during clinical examination and arterial blood gas analysis. Group 3 comprised 16 horses (4 mares and 12 geldings; 5 to 21 years old [mean,  $11.1 \pm 4.3$  years]) with mild RAO. These horses had a history of occasional coughing, especially during exercise, and mucoid nasal discharge, but they did not have a history of dyspnea. Horses were bedded on straw and fed predominantly hay but were sometimes fed grass silage. Abnormalities were detected during clinical and auxiliary examinations, but dyspnea was not detected. Group 4 comprised 11 horses (4 mares, 1 stallion, 6 geldings; 7 to 22 years old [mean,  $13.6 \pm 5.1$  years]) that had exacerbated RAO. These horses had a history of RAO and had undergone multiple environmental changes, but they currently were housed in an environment in which they were exposed to hay and straw for psychologic, practical, or economic reasons. All horses in this group had dyspnea, and other abnormalities were detected during clinical and auxiliary examinations.

**Volumetric capnography**—Volumetric capnography was performed by use of a computerized ultrasonic spirometer<sup>f</sup> as described elsewhere<sup>12</sup> and a  $CO_2$  infrared transducer (response time, 90 milliseconds).<sup>8</sup> A catheter (length, 30 cm; diameter, 1.2 mm) was attached to the ultrasonic flow sensor and used for collection of samples (aspiration rate, 200 mL/min; delay, 240 milliseconds). The capnometer was calibrated with a reference gas mixture<sup>h</sup> before measurements were obtained for each horse. To assure a specification of 2%, the ultrasonic flowmeter was calibrated with a certified calibration syringe<sup>i</sup> before and after each measurement session. Measuring procedures were performed as described elsewhere.<sup>6,7,9</sup>

Briefly, the protocol consisted of 3 or 4 measurement sessions for each horse with a mean of 22 breath cycles recorded during each session. At least 60 breath cycles of each horse were selected (end-tidal  $CO_2$ ,  $\geq 3\%$ ; deviation of inspiratory and expiratory volume, < 10%) and analyzed.<sup>6</sup> Results of volumetric capnography were adjusted on the basis of rectal temperature, air pressure, and water saturation, and the  $CO_2$  fraction of exhaled air in each breath was adjusted on the basis of the sampling delay of the  $CO_2$  analyzer before being plotted against expiratory  $V_T$ . Data for the recorded  $CO_2$  and volume curves were exported for off-line graphing of volumetric capnogram curves. Indices of pulmonary function were derived from volumetric capnography by use of specially dedicated software.<sup>1</sup>

Calculated indices included end-tidal  $CO_2$  fraction of exhaled air (FETCO<sub>2</sub>) and  $CO_2$  volume (VCO<sub>2</sub>); values for VCO<sub>2</sub> were calculated by integration of the  $CO_2$ -volume curve by use of the trapezoid method.<sup>13</sup>

Alveolar fraction of CO<sub>2</sub> (F<sub>A</sub>CO<sub>2</sub>) was calculated by use of the following equation:

$$F_{A\text{CO}_2} = (100 \times V_{\text{CO}_2}) / V_{\text{Talv}}$$

where V<sub>Talv</sub> is the alveolar portion of expiratory V<sub>T</sub>. Values for V<sub>Talv</sub> were calculated by use of the following equation:

$$V_{\text{Talv}} = V_T - V_{\text{Ds}}$$

where V<sub>Ds</sub> was the series dead-space volume. The V<sub>Ds</sub> reflects the volume contained in all pathways of the bronchial tree down to the interfaces between fresh gas and alveolar gas calculated by use of the preinterface expirate method.<sup>14</sup> The preinterface expirate method describes series dead-space as a distribution function.

Dead-space volume (V<sub>D</sub>) was calculated by use of the Bohr equation as follows:

$$V_{\text{DBohr}} = V_T - (V_T \times [\text{FECO}_2 / \text{FETCO}_2])$$

where FECO<sub>2</sub> was the mixed-expired CO<sub>2</sub> fraction. Values for FECO<sub>2</sub> were calculated by use of the following equation<sup>15</sup>:

$$\text{FECO}_2 = V_{\text{CO}_2} / V_T$$

Physiologic V<sub>D</sub> (V<sub>Dphys</sub>) was calculated by a modification of the Bohr equation, by use of the following equation<sup>16</sup>:

$$V_{\text{Dphys}} = V_T - (V_T \times [\text{FECO}_2 / \text{PaCO}_2])$$

Ratio of V<sub>D</sub> to V<sub>T</sub> was calculated for V<sub>DBohr</sub> and V<sub>Dphys</sub> (ie, V<sub>DBohr</sub>:V<sub>T</sub> and V<sub>Dphys</sub>:V<sub>T</sub>).

Alveolar dead-space volume (V<sub>Dalv</sub>) was calculated by use of the following equation:

$$V_{\text{Dalv}} = V_{\text{Dphys}} - V_{\text{Ds}}$$

Values for V<sub>Dalv</sub>:V<sub>Talv</sub> also were determined.

Values for the measured expired VCO<sub>2</sub> (A<sub>1</sub>) and the VCO<sub>2</sub> that would have been expired in an ideal lung (A<sub>2</sub>) were determined (Fig 1).<sup>9,10</sup> Values for A<sub>2</sub> were calculated by use of the following equation:

$$A_2 = (V_T - V_{\text{Ds}}) \times (\text{FETCO}_2 - A_1)$$

The ratio of A<sub>1</sub> to A<sub>2</sub> was used as an index of effective CO<sub>2</sub> elimination and was calculated by use of the following equation:

$$A_1:A_2 = (A_1 / [A_2 + A_1]) \times 100.$$

**Statistical analysis**—Descriptive procedures included calculation of mean ± SD scores of subjective clinical indices (amount and viscosity of mucus and results of cytologic examination of tracheobronchial aspirates). Coefficients of variation among the 3 blood gas samples of each horse were calculated as estimates of the relative variation of these measurements. Differences in numeric clinical indices (respiratory rate [RR], PaO<sub>2</sub>, PaCO<sub>2</sub>, and AaDO<sub>2</sub>), FETCO<sub>2</sub>, and F<sub>A</sub>CO<sub>2</sub> among groups were assessed by use of linear models and interpreted as least-squares means and 95% confidence interval (95% CI). Correlations between RR, PaO<sub>2</sub>, AaDO<sub>2</sub>, VCO<sub>2</sub>, FETCO<sub>2</sub>, F<sub>A</sub>CO<sub>2</sub>, and indices of pulmonary function derived from volumetric capnography were calculated by use of Pearson correlation coefficients. Differences of volumetric capnography indices

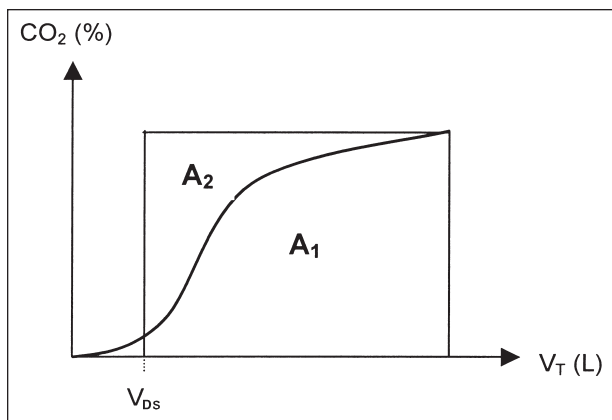


Figure 1—Figure illustrating the derivation of indices from a volumetric capnogram. The ratio of A<sub>1</sub>:A<sub>2</sub> provides an index of effective CO<sub>2</sub> elimination. A<sub>1</sub> = Measured expired volume of CO<sub>2</sub> (ie, VCO<sub>2</sub>). A<sub>2</sub> = The VCO<sub>2</sub> that would have been expired in an ideal lung. V<sub>T</sub> = Expiratory tidal volume. V<sub>Ds</sub> = Series dead-space volume.

Table 1—Clinical and auxiliary findings in healthy horses and horses with recurrent airway obstruction (RAO) with and without clinical signs of the disease

Group*	Clinical history	RR (L/min)†	Respiratory effort after exercise	Cough‡	Amount of mucus§	Viscosity of mucus	Cytologic examination¶
1 n = 21	Healthy	10 <sup>a</sup> (812)	Normal	0 and 0	1.1 ± 0.5	1.4 ± 0.7	0 ± 0
2 n = 22	Dyspnea or coughing when exposed to hay	13 <sup>a</sup> (1115)	Normal	0 and 0	2.0 ± 0.9	2.3 ± 1.1	1.3 ± 2.3
3 n = 16	Occasional coughing and mild exercise intolerance	18 <sup>b</sup> (1520)	Mildly increased	2 and 5	2.5 ± 1.0	2.8 ± 1.1	3.6 ± 3.5
4 n = 11	Dyspnea, coughing, and mild exercise intolerance	33 <sup>c</sup> (2936)	Severely increased	11 and 2	4.0 ± 0.9	4.3 ± 0.8	6.7 ± 1.4

\*Horses were assigned to groups on the basis of clinical history, respiratory flow rate (RR), respiratory effort after exercise, coughing, amount of mucus, viscosity of mucus, score of cytologic examination of tracheobronchial aspirates, and PAO<sub>2</sub> and were categorized as follows: group 1, healthy horses; group 2, horses with RAO in clinical remission; group 3, horses with mild RAO; and group 4, horses with exacerbated RAO. †Values reported are least-squares mean (95% confidence interval [CI]). ‡Values reported are number of horses coughing at rest and coughing during or after exercise. §Mean ± SD amount of tracheobronchial mucus as determined on a scale of 0 to 5 (0, isolated small blobs of mucus; 1 and 2, isolated moderate-sized blobs of mucus; 3 large blobs of mucus; 4, ventral accumulations of mucus; 5, continuous sheet of mucus). ||Mean ± SD viscosity of tracheobronchial mucus as determined on a scale of 0 to 5 (0, liquid mucus that is easily aspirated; 1, serous mucus; 2, viscous mucus that is harder to aspirate; 3 to 5, viscous mucus that is increasingly difficult to aspirate). ¶Mean ± SD values for estimated percentages of polymorphonuclear neutrophils (PMNs) in the total number of cells per microscopic field of tracheobronchial aspirates as determined on a scale of 0 to 8 (0, 0 to 10% PMNs; 1 to 4, 20 to 40% PMNs; 5 and 6, 50 to 80% PMNs; 7 and 8, 90 to 95% PMNs)

<sup>a,b,c</sup>Values with different superscript letters differ significantly (*P* < 0.05).

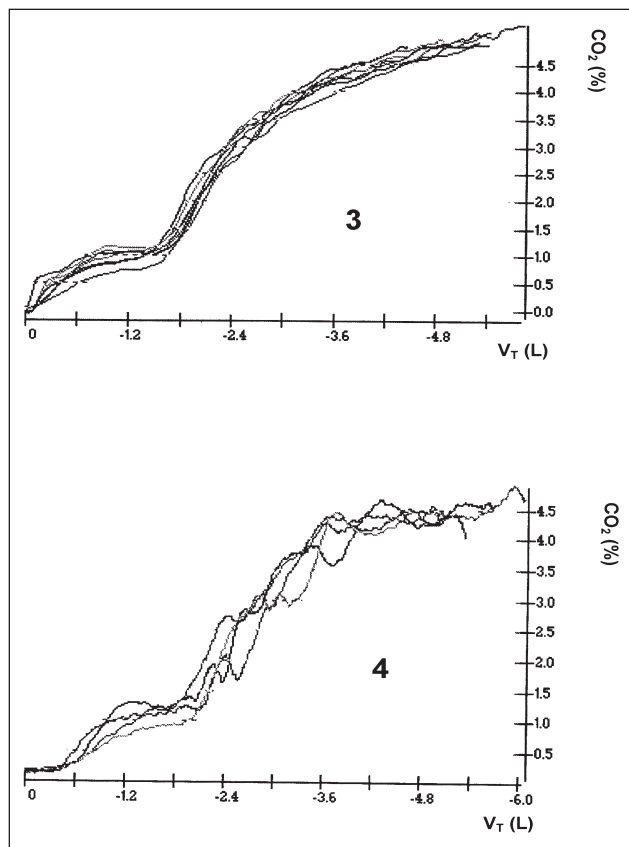
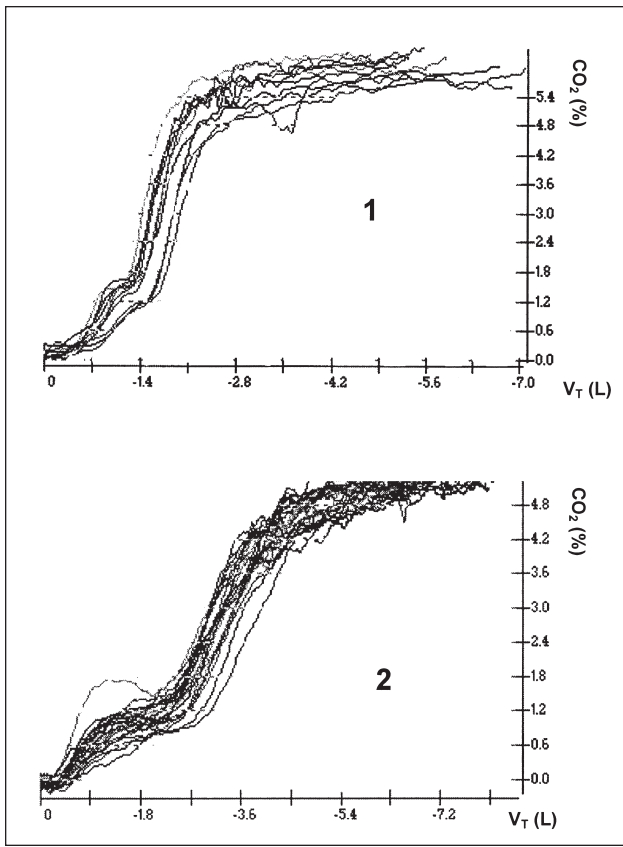


Figure 2—Volumetric capnograms of multiple expirations of a representative horse in each of 4 groups (1, healthy horse; 2, horse with recurrent airway obstruction [RAO] in remission; 3, horse with mild RAO; 4, horse with exacerbated RAO). Each line represents 1 breath; at least 60 breaths were analyzed in each horse.

among clinical groups were assessed by use of linear models and interpreted as least-squares means and 95% CI.

Selected indices of pulmonary function were defined as outcome variables for the analysis. The following model was used:

$$Y = \text{age} + \text{sex} + \text{session} + \text{breath} + \text{group} + \text{use} + (\text{group} \times \text{sex}) + (\text{group} \times \text{age}) + (\text{group} \times \text{use})$$

where Y was the outcome variable ( $V_T$ ,  $V_{CO_2}$ ,  $V_{DBohr}$ : $V_T$ ,  $V_{Dphys}$ : $V_T$ ,  $V_{Dalv}$ : $V_{Talv}$ ,  $A_1$ : $A_2$ ), age was number of years, sex was male or female, session was number of the measurement session, breath was the number of the breath within the measurement session, group was clinical group (1 to 4), use was activity of the horse prior to entry into the study (pleasure riding or sporting events [dressage or show jumping]), and the interaction terms.

Multiple comparisons among clinical groups were performed by use of the Bonferroni procedure. Agreement between observed data and models was assessed by analysis of residuals and variance of outcome variables accounted for by the models ( $R^2$  values). Power calculations of statistical tests were performed for comparisons of clinical groups by use of a type-I error rate of 0.05. All analyses were performed by use of a statistical computer program.<sup>k</sup> Significance was defined as values of  $P \leq 0.05$  for all analyses.

## Results

**Clinical and auxiliary findings**—Coefficient of variation among the 3 blood gas samples of each horse was 1.8% for  $PaO_2$  and 1.7% for  $PaCO_2$ . Clinical findings and scores of subjective clinical indices were

Table 2—Least-squares mean (95% CI) values for results of arterial blood gas analysis, end-tidal  $CO_2$  fraction ( $F_{ETCO_2}$ ), and alveolar  $CO_2$  fraction ( $F_A CO_2$ ) in healthy horses and horses with RAO with and without clinical signs of disease

Group*	$PaO_2$ (mm Hg)	$PaCO_2$ (mm Hg)	$AaDO_2$ (mm Hg)	$F_{ETCO_2}$ (%)	$F_A CO_2$ (%)
1 n = 21	100.0 <sup>a</sup> (96.6–103.5)	39.0 <sup>a</sup> (38.1–39.8)	2.3 <sup>a</sup> (–1.2–[–5.8])	5.4 <sup>a</sup> (5.1–5.7)	3.8 <sup>a</sup> (3.5–4.1)
2 n = 22	95.6 <sup>a</sup> (91.9–99.2)	41.0 <sup>b</sup> (40.1–41.9)	5.2 <sup>b</sup> (1.5–8.9)	5.3 <sup>a</sup> (5.0–5.5)	3.8 <sup>a</sup> (3.5–4.1)
3 n = 16	84.9 <sup>b</sup> (81.0–88.9)	41.5 <sup>b</sup> (40.5–42.5)	15.2 <sup>b</sup> (11.2–19.2)	5.2 <sup>a</sup> (4.9–5.5)	3.7 <sup>a</sup> (3.3–4.0)
4 n = 11	69.6 <sup>c</sup> (64.1–75.1)	42.0 <sup>b</sup> (40.6–43.3)	29.6 <sup>c</sup> (24.0–35.1)	4.3 <sup>b</sup> (3.9–4.7)	3.0 <sup>b</sup> (2.6–3.4)

$AaDO_2$  = Difference in  $PAO_2$  and  $Pao_2$ .  
See Table 1 for remainder of key.

Table 3—Least-squares mean (LS mean) and 95% CI for expiratory tidal volume ( $V_T$ ) and expired volume of  $CO_2$  ( $V_{CO_2}$ ) in healthy horses and horses with RAO with and without clinical signs of the disease

Group*	$V_T$ (L)		$V_{CO_2}$ (mL)	
	LS mean	95% CI	LS mean	95% CI
1	5.6 <sup>a</sup>	5.4–5.9	175.8 <sup>a</sup>	163.9–186.6
2	5.3 <sup>b</sup>	5.1–5.5	137.0 <sup>b</sup>	126.7–148.7
3	4.9 <sup>c</sup>	4.7–5.1	117.6 <sup>c</sup>	107.4–127.8
4	4.7 <sup>d</sup>	4.5–4.9	49.2 <sup>d</sup>	49.2–69.4

<sup>a,b,c,d</sup> Within a column, values with different superscript letters differ significantly ( $P < 0.05$ ).  
See Table 1 for remainder of key.

Table 4—Values (LS mean and 95% CI) for indices derived from volumetric capnography in healthy horses and horses with RAO with and without clinical signs of the disease

Group*	VD <sub>Bohr</sub> :V <sub>T</sub>		VD <sub>phys</sub> :V <sub>T</sub>		VD <sub>alv</sub> :V <sub>Talv</sub>		A <sub>1</sub> :A <sub>2</sub> (%)	
	LS mean	95% CI	LS mean	95% CI	LS mean	95% CI	LS mean	95% CI
1	0.48 <sup>a</sup>	0.46–0.50	0.52 <sup>a</sup>	0.49–0.54	0.39 <sup>a</sup>	0.37–0.42	56.5 <sup>a</sup>	54.8–58.1
2	0.53 <sup>b</sup>	0.52–0.55	0.58 <sup>b</sup>	0.56–0.61	0.40 <sup>b</sup>	0.38–0.42	51.1 <sup>b</sup>	49.6–52.7
3	0.57 <sup>b</sup>	0.56–0.59	0.64 <sup>c</sup>	0.62–0.66	0.43 <sup>c</sup>	0.41–0.45	48.9 <sup>c</sup>	47.4–50.3
4	0.69 <sup>c</sup>	0.67–0.70	0.75 <sup>d</sup>	0.73–0.77	0.48 <sup>d</sup>	0.46–0.50	40.8 <sup>d</sup>	39.3–42.2

VD<sub>Bohr</sub> = Dead-space volume (Vd) calculated by use of the Bohr equation. VD<sub>phys</sub> = Physiologic Vd. VD<sub>alv</sub> = Alveolar Vd. A<sub>1</sub>:A<sub>2</sub> = Index of effective CO<sub>2</sub> elimination. A<sub>1</sub> = Measured expired VCO<sub>2</sub>. A<sub>2</sub> = VCO<sub>2</sub> that would have been expired in an ideal lung. See Table 1 for remainder of key.

Table 5—Significant correlations between selected variables for pulmonary function and volumetric capnography indices in healthy horses and horses with RAO with and without clinical signs of the disease

Variable	RR	PaO <sub>2</sub>	AaDO <sub>2</sub>	VCO <sub>2</sub>	FETCO <sub>2</sub>	F <sub>A</sub> CO <sub>2</sub>
RR	—	-0.7	0.69	-0.33	-0.41	-0.33
PaO <sub>2</sub>	-0.7	—	0.98	0.29	0.29	0.27
AaDO <sub>2</sub>	0.69	0.98	—	-0.33	-0.32	-0.29
VCO <sub>2</sub>	-0.33	0.29	-0.33	—	0.82	0.74
FETCO <sub>2</sub>	NS	NS	NS	0.82	—	0.85
F <sub>A</sub> CO <sub>2</sub>	NS	NS	NS	0.74	0.85	—
V <sub>T</sub>	NS	0.32	-0.33	0.73	0.40	0.36
VD <sub>Bohr</sub> :V <sub>T</sub>	0.33	-0.30	0.36	-0.86	-0.77	-0.76
VD <sub>phys</sub> :V <sub>T</sub>	0.39	-0.35	0.40	-0.88	-0.86	-0.81
VD <sub>alv</sub> :V <sub>Talv</sub>	0.32	-0.32	0.33	-0.61	-0.69	-0.91
A <sub>1</sub> :A <sub>2</sub>	-0.25	NS	-0.28	0.83	0.70	0.73

— = Not applicable. NS = Correlation was not significant at a value of P < 0.05. See Tables 2, 3, and 4 for key.

determined for groups 1 to 4 (Table 1). Results of numeric clinical and auxiliary data were reported as least-squares mean and 95% CI (Table 2). Values for RR, PaO<sub>2</sub>, and AaDO<sub>2</sub> differed significantly for groups 1 and 2, compared with values for groups 3 and 4. The PaCO<sub>2</sub> for group 1 differed significantly from the PaCO<sub>2</sub> for groups 2, 3, and 4. Least-squares means of FETCO<sub>2</sub> and F<sub>A</sub>CO<sub>2</sub> for group 4 differed significantly from values for FETCO<sub>2</sub> and F<sub>A</sub>CO<sub>2</sub> for groups 1, 2, and 3.

**Results of volumetric capnography**—Results of volumetric capnography were recorded for each horse (Fig 2). Power calculations for the effect of group revealed values > 0.95 for all indices included in the study. Results of V<sub>T</sub> and VCO<sub>2</sub> were reported as least-squares mean and corresponding 95% CI (Table 3). Least-squares mean values of V<sub>T</sub> and VCO<sub>2</sub> differed significantly among groups. Least-squares means and 95% CI of indices of pulmonary function derived by use of volumetric capnography were determined (Table 4). Least-squares mean values for VD<sub>Bohr</sub>:V<sub>T</sub>, VD<sub>phys</sub>:V<sub>T</sub>, and A<sub>1</sub>:A<sub>2</sub> differed significantly among groups. Least-squares mean values of VD<sub>alv</sub>:V<sub>Talv</sub> differed significantly between groups 1 and 3 and groups 1 and 4, but they did not differ significantly between groups 1 and 2.

Except for sex, explanatory variables had a significant effect on V<sub>T</sub>, VCO<sub>2</sub>, VD<sub>Bohr</sub>:V<sub>T</sub>, VD<sub>phys</sub>:V<sub>T</sub>, VD<sub>alv</sub>:V<sub>Talv</sub>, and A<sub>1</sub>:A<sub>2</sub>. The interaction terms, group × sex and age × use, also had a significant effect on all indices in the study. Analysis of graphs of residuals revealed a normal distribution for linear models. Fit of

the models was considered to be satisfactory (R<sup>2</sup> values ranged from 0.2 to 0.3). We detected significant correlation coefficients between RR, PaO<sub>2</sub>, AaDO<sub>2</sub>, VCO<sub>2</sub>, FETCO<sub>2</sub>, and F<sub>A</sub>CO<sub>2</sub>, and volumetric capnography indices (Table 5).

## Discussion

In the study reported here, volumetric capnography indices V<sub>T</sub>, VCO<sub>2</sub>, VD<sub>Bohr</sub>:V<sub>T</sub>, VD<sub>phys</sub>:V<sub>T</sub>, and A<sub>1</sub>:A<sub>2</sub> identified deficits in pulmonary function in horses with RAO in remission (group 2), horses with mild RAO (group 3), and horses with exacerbated RAO (group 4). Analysis of these results suggests that volumetric capnography is more sensitive than the pneumotachograph-esophageal balloon method, which has been used to detect deficits in pulmonary function in horses with exacerbated RAO but not horses with mild RAO or horses with RAO in remission.<sup>1,3</sup> In comparison to clinically normal horses, horses with RAO have an increased scattering of values for V<sub>A</sub>/Q, and the magnitude of V<sub>A</sub>/Q inequality is correlated with clinical signs and severity of bronchiolitis.<sup>17,18</sup> Regional variation in ventilation per unit of perfusion produces a spectrum of values for V<sub>A</sub>/Q and causes dead space. Uneven gas distribution and incomplete diffusion are the most important causes of dead space, whereas temporal V<sub>A</sub>/Q mismatching and venous admixture are, as a rule, less important.<sup>19</sup> The VD<sub>phys</sub> is believed to be a reliable index of total dead-space ventilation in humans and includes the alveolar dead-space fraction.<sup>8,20</sup>

The CO<sub>2</sub> washout profile, represented by the indices VCO<sub>2</sub> and A<sub>1</sub>:A<sub>2</sub> in the study reported here, differed significantly between all groups of horses (Tables 4 and 5). The index A<sub>1</sub>:A<sub>2</sub> is a measure of effective CO<sub>2</sub> elimination, and it compares the measured expired VCO<sub>2</sub> with the VCO<sub>2</sub> that would have been expired in an ideal lung. In another study,<sup>21</sup> our laboratory group documented that the shape of volumetric capnograms differs in horses depending on the degree of airway obstruction. The index A<sub>1</sub>:A<sub>2</sub> reflects the shape of the volumetric capnogram,<sup>1</sup> and it has the advantage of being expressed as a percentage of the function expected from a perfect lung. A rectangular-shaped volumetric capnogram would have an efficiency of 1.0. Low values of A<sub>1</sub>:A<sub>2</sub> are usually attributable to an increase in alveolar dead space, but it can also be caused by an increase in airway dead space.<sup>1</sup>

In contrast to VD<sub>alv</sub>:V<sub>Talv</sub>, the VD<sub>Bohr</sub>:V<sub>T</sub>, VD<sub>phys</sub>:V<sub>T</sub>, and A<sub>1</sub>:A<sub>2</sub> could be used to detect pulmonary dysfunction.

tion in RAO-affected horses without clinical signs. These 3 volumetric capnography indices all include airway dead space, whereas  $V_{\text{dalv}}:V_{\text{Talv}}$  does not. In another study,<sup>22</sup> it was determined that airway dead space also depends on the stiffness of the airways and that the change of airway dead space with lung volume during the breathing cycle is reduced in mild asthmatic human patients, compared to nonasthmatic subjects. Investigators of that study speculated that this finding was a consequence of structural changes or remodeling associated with airway inflammation, which is a feature of asthma. Although speculative, we suggest that remodeling associated with airway inflammation contributes to the ability of the indices  $V_{\text{DBohr}}:V_{\text{T}}$ ,  $V_{\text{Dphys}}:V_{\text{T}}$ , and  $A_1:A_2$  to detect airway obstruction when the disease is not active. Values for  $V_{\text{DBohr}}:V_{\text{T}}$  reflect global ventilatory efficiency, which includes the airway dead-space fraction. In the clinically normal horses reported here, nearly half (48%) of total ventilation was dead-space ventilation, as determined by use of the Bohr equation, and dead-space ventilation represented 69% of total ventilation in horses with exacerbated RAO (Table 5), which is slightly lower than the value reported in another study.<sup>17</sup> Whether  $\text{PCO}_2$  of the end-tidal portion of an expired breath is equal or approximately equal to  $\text{PaCO}_2$  depends on the magnitude of the alveolar dead space and the spread of values for  $\dot{V}/\dot{Q}$ . Therefore, dead space determined in accordance with the Bohr equation is underestimated in relation to the  $V_{\text{Dphys}}$  in most cases, which is consistent with our results.

The  $V_{\text{dalv}}:V_{\text{Talv}}$  index differentiated between healthy horses and horses with mild RAO or horses with exacerbated RAO (Table 5). Alveolar dead space is believed to be an index of the uniformity of blood flow distribution to the lungs<sup>23</sup> and is a sensitive measure for use in predicting pulmonary embolisms in humans.<sup>24</sup> In the study reported here,  $V_{\text{dalv}}:V_{\text{Talv}}$  was not able to differentiate between healthy horses and horses with RAO in clinical remission. Perfusion pattern of the lungs may not be substantially altered in RAO-affected horses that do not have clinical signs of the disease. On the other hand, horses with mild RAO or exacerbated RAO had an increase in alveolar dead space, indicating uneven peripheral perfusion. In another study,<sup>9</sup> an increase in  $V_{\text{dalv}}:V_{\text{Talv}}$  was associated with high apparent mucus viscosity. We speculated that in horses with clinically apparent RAO, mechanisms that predispose to high apparent mucus viscosity result in mucus plugs in small airways, which could directly lead to uneven airflow distribution in the periphery. The alveolar-vascular reflex decreases perfusion of hypoventilated alveoli (hypoxic vasoconstriction), and blood is distributed to normal or hyperventilated alveoli.<sup>25</sup>

The 95% CI of  $V_{\text{T}}$  differed significantly among all groups of horses. Values reported here were similar to those reported in another study<sup>3</sup>; however, in that other study,  $V_{\text{T}}$  differed significantly only between RAO-affected horses in crisis and healthy control horses but not between horses with RAO in remission and control horses. The larger number of horses in our study resulted in an improvement in statistical power ( $> 0.95$ ). Furthermore, we averaged measurements during a period of at least 60 breath cycles, which will assure good repeatability,<sup>6,7</sup> compared with values obtained for only

10 selected breaths.<sup>3</sup> Also, the multivariate statistical model we used accounted for significant effects of age and use of the horses on pulmonary function. Although the multivariate statistical model used explained only 20 to 30% of the total variance, these effects and the complex interaction terms may obscure disease-related differences in pulmonary function of the horses when these effects are not considered during interpretation of results of pulmonary function tests. Furthermore, results of volumetric capnography were reported as least-squares means and 95% CIs. The latter avoid the use of a single cutoff point for determining significant differences, because the choice of such a cutoff point is arbitrary at best. The 95% CIs around our mean results provide a 95% chance that the real difference lies between the upper and lower limits of the 95% CI.

Many asthmatic patients, including children and adults, have evidence of residual airway obstruction<sup>26</sup> despite a lack of clinical signs.<sup>27</sup> Peripheral lung resistance can be increased in people who are asymptomatic asthmatics with normal forced expiratory volume in 1 second ( $\text{FEV}_1$ ).<sup>28</sup> Furthermore,  $\text{FEV}_1$  has not been related to substantial unevenness of ventilation detected by analysis of nitrogen washout curves in humans with mild stable asthma,<sup>29</sup> and  $\text{FEV}_1$  alone has not been able to define remission adequately or clearly identify those patients prone to deterioration of gas exchange.<sup>30</sup> Couetil et al<sup>3</sup> reported that  $\text{FEV}_1$  was also of limited value in horses. Variables reflecting airflow during the late phase of forced expiration and that document obstruction originating in the distal airways, however, can discriminate between healthy horses and horses with RAO in remission or horses with mild RAO.<sup>3</sup>

Furthermore, Hoffman et al<sup>3</sup> documented that forced oscillation combined with histamine bronchoprovocation is a sensitive method to identify deficits of pulmonary function in horses with mild obstruction of the bronchioles and alveoli. Those findings and the results of the study reported here clearly reveal that various tests of pulmonary function can detect airway obstruction in horses with mild, even clinically inapparent, respiratory tract disease. However, in a study by Couetil et al,<sup>3</sup> horses with RAO in remission had more severe airflow obstruction than horses with mild RAO. The opposite was the case in the study reported here. Although this difference may simply be attributable to differing historical and clinical selection criteria for the study groups, it is interesting to speculate on possible pathophysiologic causes for the discrepancy. Use of sedation for measurements of forced expiration in that other study<sup>3</sup> may have decreased the bronchospastic component of airflow obstruction attributable to smooth muscle relaxation. The horses in our study were not sedated. Furthermore, dead-space indices may have detected increased  $\dot{V}_A/\dot{Q}$  inequality attributable to altered perfusion patterns evident in horses with mild RAO but not in horses with RAO in remission.

Arterial blood gas analysis is much more commonly available than any pulmonary function test. The  $\text{AaDO}_2$  has been used to assess  $\dot{V}_A/\dot{Q}$  mismatching in horses with RAO.<sup>2</sup> However, in the study reported here,  $\text{AaDO}_2$  and  $\text{PaO}_2$  did not discriminate between groups 1

and 2, and  $P_{aCO_2}$  did not discriminate among groups 2, 3, and 4 (Table 2). Partial  $\dot{V}_A/\dot{Q}$  mismatching in horses with mild RAO is most likely not identified by arterial blood gas analysis because of the aforementioned alveolar-vascular reflex.<sup>26</sup> Values for  $V_{CO_2}$ ,  $F_{ETCO_2}$ , and  $F_{ACO_2}$  were significantly and strongly inversely correlated ( $r$ , 0.61 to 0.91) with values for dead-space indices. Correlation coefficients between  $AaDO_2$  or  $PaO_2$  and dead-space indices were significant, but they were only weakly correlated ( $r$ , 0.28 to 0.40). In another study,<sup>9</sup> our laboratory group found similar weak correlations ( $r$ , 0.28 to 0.35) between  $PaO_2$  and volumetric capnography indices in all horses of that study population, whereas in distinct groups of horses with varying degrees of airway obstruction, we did not detect correlations between  $PaO_2$  and volumetric capnography indices. Human asthma patients with similar values for arterial blood gases often have dissimilar  $\dot{V}_A/\dot{Q}$  patterns, as estimated by use of multiple elimination techniques for inert gases. Consequently, the pattern of  $\dot{V}_A/\dot{Q}$  inequality cannot be inferred from arterial  $PaO_2$ .<sup>31</sup> The  $AaDO_2$  is the difference in oxygen tension between an ideal alveolus and an ideal artery with the uniform  $\dot{V}_A/\dot{Q}$  of the lungs as a whole, calculated on the basis of  $P_{aCO_2}$ , the respiratory exchange ratio, and barometric pressure. Most important, causes of dead-space ventilation are reflected by mismatching on both sides of the gas exchange interface.<sup>8</sup> Alveolar dead space detects mismatching between and within terminal units of the lungs, which can be time-dependent or time-independent factors.<sup>8</sup> These observations correspond to the finding that  $PaO_2$  and  $AaDO_2$  were significantly but only weakly correlated with volumetric capnography indices in healthy horses and horses with RAO. Partial disturbances of a uniform  $\dot{V}_A/\dot{Q}$  during early stages of airway obstruction in horses with RAO lead to decreased perfusion of hypoventilated alveoli, which creates dead space detectable by volumetric capnography indices but not by  $PaO_2$  and  $AaDO_2$ .

Volumetric capnography indices provide indices of the existence of  $\dot{V}_A/\dot{Q}$  mismatching in horses with clinically apparent RAO as well as horses with RAO in remission that do not have clinical signs of the disease. The  $V_{DBohr}:V_T$  and  $V_{Dphys}:V_T$ , which served as indices of wasted ventilation, and  $A_1:A_2$ , which is an indicator of effective  $CO_2$  elimination from the lungs, were sufficiently sensitive measures to distinguish between healthy horse and RAO-affected horses that did not have clinical signs of the disease. To optimize the ability of volumetric capnography indices to differentiate among groups of horses in a heterogenous population, it is important to account for the significant effects of age and use of the horses.

<sup>a</sup>Moens YPS. *Ventilation and gas exchange in each lung of the anaesthetised horse. The influence of body position and mechanical ventilation*. PhD Thesis, Department of Anesthesiology, University of Utrecht, Netherlands, 1992.

<sup>b</sup>Lidocaine 2%, Chassot, Belp, Switzerland.

<sup>c</sup>Classic digital thermometer, Polylabo SA, Berne, Switzerland.

<sup>d</sup>840 Ciba Corning blood gas analyzer, Ciba Corning Diagnostics Corp, Medfield, Mass.

<sup>e</sup>Certified mercury barometer, Büchi AG, Bern, Switzerland.

<sup>f</sup>Ultrasonic spirometer, Isler Bioengineering AG, Zürich, Switzerland.

<sup>g</sup>IR 1507 miniature fast-response  $CO_2$  infrared transducer, Servomex, Jarvis Brook, Crowborough, UK.

<sup>h</sup>Quick Cal calibration gas 5%  $CO_2 \pm 0.5\%$  relative, Datex Ohmeda Division, Helsinki, Finland.

<sup>i</sup>7-L calibration syringe, Hans Rudolph Inc, Kansas City, Mo.

<sup>j</sup>SBD- $CO_2$ calc, Oberli-Engineering, Berne, Switzerland.

<sup>k</sup>SAS, release 8.2, SAS Institute Inc, Cary, NC.

<sup>l</sup>Fletcher R. *The single breath test for carbon dioxide*. PhD Thesis, Department of Anaesthesia and Clinical Physiology, University of Lund, Berlings, Arlöv, Schweden, 1980.

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