Reprinted from the AMERICAN JOURNAL OF VETERINARY RESEARCH, Vol. 45, No. 2, Pages 342-345.

Pulmonary function testing in calves: Technical data

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SUMMARY

Measurements of airflow ($\dot{V}$), tidal volume (V_t), and intrapleural pressure (Ppl) were tested for accuracy in 5 healthy Dutch Friesian calves with an average body weight of 153 kg. A face mask was constructed, using fiberglass and polyester, taking into account the typical facial morphology of the calf. It was tested for airtightness, dead space, laminarity of the expiratory $\dot{V}$, and absence of saliva into the pneumotachograph. Three different systems for measuring Ppl (pleura puncturing, esophageal balloon catheter, and esophageal saline solution-filled catheter) were tested in vitro, in a Woulfe's flask, and in vivo, in the 5 calves previously described. Moreover, Ppl measured at 3 different puncture sites of the thorax and at 3 different thoracic positions of the esophagus were compared.

The frequency-response was flat to 5 Hz for the intrapleural needle and the balloon catheter, but not for the saline solution-filled catheter. The pulmonary function values obtained by puncture of the pleura at the right 9th intercostal space on a line running from the tuber coxae to the shoulder joint and by the esophageal balloon catheter, the balloon being positioned between the crossing point with the aorta and the 2 largest caudal mediastinal lymph nodes, did not differ significantly. The Ppl changes during normal breathing were greatest in the ventral site of the thorax and in the caudal thoracic portion of the esophagus, less in the dorsal site of the thorax and the middle thoracic portion of the esophagus, and least in the cranial site of the thorax and the cranial thoracic portion of the esophagus. When the balloon was positioned between the crossing point with the aorta and the 2 largest caudal mediastinal lymph nodes, Ppl recordings showed a twice smaller variability than with the 2 other esophageal positions.

Pulmonary function measurements in resting nonanesthetized animals are largely based on simultaneous recordings of intrapleural pressure (Ppl), respiratory airflow ($\dot{V}$), and tidal volume (V_t).[1-3] Despite the high frequency of respiratory tract problems in calves, little information is available about the mechanics of breathing in these animals[4-6] and was obtained by methods transposed from man and horse. These species, however, substantially differ from the calf with regards to respiratory anatomy, physiology, and behavior.[7] The purpose in the present study, therefore, was to determine the most suitable methods for measuring Ppl, $\dot{V}$, and V_t in nonanesthetized and unsedated standing calves.

Materials and Methods

Five healthy male Dutch Friesian calves between 5 to 6 months of age and weighing between 148 to 164 kg were used. They were kept in the same room and fed with a normal hay and concentrates ration. All measurements were done in a resting nonanesthetized and unsedated state in an airconditioned experimental room (18 C, barometric pressure 770 Torr). Respiratory $\dot{V}$ was measured, using a Fleisch pneumotachograph (N°3)[a], and V_t was derived electronically by integrating $\dot{V}$ with respect to time. A breathing mask was constructed, using as model the muzzle of a dead calf with body size similar to the experimental calves (Fig 1). The muzzle was covered by 2 layers (3 mm) of high-density fiberglass (450 g/m^2)[b] which was uniformly impregnated by a polyester solution[c] previously mixed with a catalysator.[d] After solidification, the mask was separated from its model and a small extension was constructed to allow an airtight coaptation with the pneumotachograph.

A rubber joint was used to assure an airtight junction between the muzzle and the posterior opening of the mask. Rubber foam was glued inside the caudal and ventral portion of this mask to insure better comfort. The airtightness and the dead space of the mask, ie, the volume of the mask which surrounds the muzzle, were checked by the closed-circuit helium dilution method.[8] The mask was placed upon the muzzle of the dead calf and the nares and the mouth were maintained airtight by using plastic tapes. The anterior opening of the mask was connected to the closed spirometer circuit[e] containing a known quantity of helium. Knowing the initial spirometer volume and the initial and final helium concentration, the added volume of air, ie, the dead space of the face mask, was calculated after the helium equilibration. The influence on respiration by the mask was tested in the 5 calves by comparing the Ppl and arterial gas values before and at various moments after placing. The mask was also frequently verified for absence of saliva into the pneumotachograph.

The space between the nares and the pneumotachograph was reduced to a minimum to avoid excessive dead space in the mask. Therefore, the laminarity of the expiratory $\dot{V}$ was checked by measuring the $\dot{V}$ simultaneously with 2 pneumotachographs put in series. The expiratory $\dot{V}$ being necessarily laminar after passage through the proximal pneumotachograph, it was postulated that

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Received for publication May 31, 1983.

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The authors thank Dr. Geubelle, Dr. Oyaert, Dr. Bienfet, and their coworkers for advice.

[a] Gould Godart, Bilthoven, The Netherlands.

[b] Polyservice, Arkel, The Netherlands.

[c] PS 28, polyservice, Arkel, The Netherlands.

[d] Mek P, polyservice, Arkel, The Netherlands.

[e] Pulmonet, Gould Godart, Bilthoven, The Netherlands.

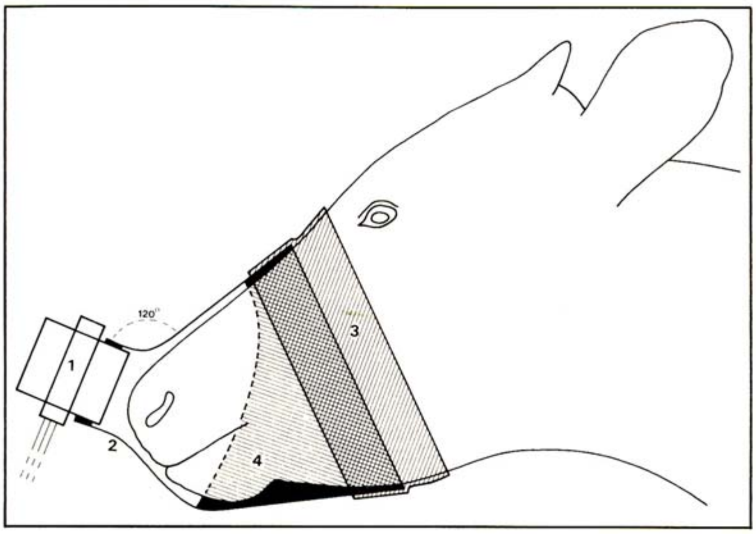

Fig 1—Diagram of pneumotachograph-mask assembly on calf. 1 = pneumotachograph; 2 = fiberglass portion of the mask; 3 = rubber portion of the mask; 4 = rubber foam.

TABLE 1— Influence of the dead space of the breathing apparatus on some respiratory and blood gas values in three healthy Friesian calves

	Dead space					
	0 ml (no mask)		220 ml*		440 ml*	
Item	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Resp frequency	25[a]	5	23[a]	4	29[a]	6
MaxdPpl (kPa)	0.70[a]	0.10	0.72[a]	0.12	0.80[b]	0.15
Pa_{O_2} (kPa)	12.3[a]	1.6	12.1[a]	1.5	10.8[b]	1.8
Pa_{CO_2} (kPa)	5.1[a]	0.4	5.2[a]	0.2	5.8[b]	0.6

* Values measured 5 minutes after placing the mask.

Only data affected of different letters are significantly different (Student's *t* test for paired data; $P \leq 0.05$).

TABLE 2—Comparison of three Ppl measuring methods in five healthy calves

	Pleura puncturing		Balloon catheter*		Saline solution-filled catheter*	
Item	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Min Ppl (kPa)	−1.26[a]	0.19	−1.24[a]	0.14	...†	...†
Max Ppl (kPa)	−0.57[a]	0.11	−0.52[a]	0.09	...†	...†
Maxd Ppl (kPa)	0.70[a]	0.09	0.72[a]	0.05	0.79[b]	0.09
C_1dyn (1/kPa)	3.57[a]	0.80	3.67[a]	0.20	3.06[a]	0.60
R_1 (kPa/1/s)	0.19[a]	0.03	0.18[a]	0.04	0.22[b]	0.02
Wvis (J)	0.57[a]	0.11	0.60[a]	0.08	0.65[b]	0.12

* Catheter tip at 80 cm from the nares; † absolute values were not recorded because of the presence of the water column in this system.

Only data affected of different letters are significantly different (Student's *t* test for paired data; $P \leq 0.05$).

the expiratory $\dot{V}$ at the exit of the mask was sufficiently laminar were the latter expiratory $\dot{V}$ (recorded by the proximal pneumotachograph) not different from the $\dot{V}$ recorded by the distal one.

The influence of the dead space of the external breathing apparatus (the face mask plus the pneumotachograph) on some pulmonary and blood gas values was measured by increasing this dead space with a series of tubes added between the anterior opening of the mask and the pneumotachograph. Arterial blood was sampled from a catheter introduced in the a. saphena and was analyzed immediately, using a blood gas analyzer[f] by the method described by Kiorpes et al.[5]

Three different Ppl measuring systems were tested for accuracy. First, a direct system introducing a pneumothorax needle[g] (L:10 cm; ID:3 mm) with 2 side holes near the tip into the pleural space. Secondly, an indirect system introducing a catheter (L:160 cm; ID:3 mm) in the thoracic portion of the esophagus. The tip of this catheter was covered by a thin latex balloon (L:9 cm; per:5 cm; thick:0.01 mm). Thirdly, an open-tip saline solution-filled catheter (L:160 cm; ID:3 mm) also introduced in the thoracic portion of the esophagus.

These 3 measuring systems were tested in vitro, using a Woulfe's flask with 4 openings.[9] One opening was connected to a pump giving a quasisinusoidal signal and a 2nd was connected to the pneumothorax needle. Through the 3rd and 4th openings, the balloon catheter and the saline solution-filled catheter were introduced into the flask, respectively. The needle and the 2 catheters were connected to 3 identical electromanometers.[h] The 3 signals were recorded simultaneously on a polygraph.[i] These 3 measuring systems were tested for giving a flat frequency response to 5 Hz, using pressure variations up to 5 kPa. Frequency-response characteristics of the pneumotachograph and the Ppl recording systems were matched up to 5 Hz.[10]

The 3 Ppl measuring systems also were compared in the 5 calves by simultaneous recording of intrapleural and intraesophageal pressure — the latter being measured with the balloon catheter and with the saline solution-filled catheter. After clipping and cleaning the puncture site, the intrapleural needle was introduced into the right 9th intercostal space on an imaginary line running from the tuber coxae to the shoulder joint. The 2 esophageal catheters were introduced via the nose in the caudal third of the esophagus. Using radiographic examination, the tips of these catheters were positioned 5 cm caudodorsal to the trachea bifurcation, 80 cm from the nares.

The compliance of the balloon wall was tested by injecting air into the balloon at ambient pressure through a 3-way stopcock connected to a syringe and an electromanometer. A pressure/volume diagram was so plotted for each balloon.

To avoid signal distortion due to the elasticity of the esophageal wall, the minimum volume of air was introduced into the balloon, care being taken to stay within the ranges of high compliance of the balloon walls. The open-tip catheter was filled with saline solution. Care was taken to avoid any leakage, obstruction, and air/water mixture in the different manometric systems.

The 3 Ppl measurements, $\dot{V}$, and V_t of each calf were recorded simultaneously. The mean pulmonary function values were derived from measurement of 5 normal respiratory cycles. Only data from 10 successive regular and artifact-free cycles were analyzed.

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[f] AVL 93750, Schaffhausen, Switzerland.

[g] Acufirm tissu canula, 1498. Ernst Kratz, Dreieich, West Germany.

[h] Statham Instruments, p23Db, Gould Godart, Bilthoven, The Netherlands.

[i] Brush 2600, Gould Godart, Bilthoven, The Netherlands.

TABLE 3—Comparison of Ppl values for different positions of the esophageal balloon catheter in five healthy calves

	Position of the catheter tip					
	70 cm from the nares		80 cm from the nares		90 cm from the nares	
Item	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Min Ppl (kPa)	-1.07^a	0.19	-1.24^b	0.14	-1.24^b	0.29
Max Ppl (kPa)	-0.52^a	0.13	-0.52^a	0.09	-0.39^b	0.15
Maxd Ppl (kPa)	0.55^a	0.12	0.72^b	0.05	0.85^c	0.08

Only data affected of different letters are significantly different (Student's *t* test for paired data; $P \leq 0.05$).

TABLE 4—Comparison of Ppl values for different sites of pleura puncturing in five healthy Friesian calves

	Cranial position		Dorsal position		Ventral position	
Item	$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
Min Ppl (kPa)	-1.31^a	0.31	-1.32^a	0.15	-1.16^b	0.24
Max Ppl (kPa)	-0.67^a	0.12	-0.61^b	0.09	-0.40^c	0.12
Maxd Ppl (kPa)	0.65^a	0.15	0.71^b	0.09	0.77^c	0.09

Only data affected of different letters are significantly different (Student's *t* test for paired data; $P \leq 0.05$).

Before each test, the electromanometers were calibrated with a mercury manometer and the pneumotachograph with a flow calibration set.[j] All lung volumes were corrected to body temperature, pressure saturated units.

For the 3 Ppl measuring methods, the values of lowest Ppl during inspiration (min Ppl), highest Ppl during expiration (max Ppl), peak-to-peak Ppl change (maxd Ppl), dynamic lung compliance ($C_t$dyn), total pulmonary resistance (R_t), and viscous work of breathing (Wvis) were compared. The last 3 values were calculated, using the method described by Geubelle.[11]

To establish the optimal position of the tip of the intraesophageal catheter, Ppl values recorded at different sites between the cardiac area and the cardia were compared by simultaneous recordings of Ppl with 2 identical balloon catheters, the tip of the 1st one being positioned at 80 cm from the nares and the tip of the 2nd being positioned respectively at 70 and 90 cm from the nares. Moreover, Ppl values recorded simultaneously at 3 different puncture sites were compared in 5 other Friesian calves of the same body size as those described. The 1st needle was introduced into the right 9th intercostal space on a line running from the tuber coxae to the shoulder joint (dorsal position), the 2nd, in the 8th intercostal space 10 cm lower than the 1st one (ventral position), and the 3rd, in the 7th intercostal space at the same horizontal level as the 1st (cranial position).

Results

The dead space of the breathing apparatus was approximately 220 ml. This represents 15% of the mean V_t (1,500 ml ± 60) of the 5 calves used in this study. Because of the fact that complete helium equilibration was rapidly observed in the closed spirometer circuit, it was concluded that the face mask positioned upon the muzzle was airtight. Saliva discharge to the pneumotachograph was not observed, in spite of constant ptyalism inside the mask.

A temporary increase in Ppl variations was observed when placing the mask. Nevertheless, this had disappeared entirely after a period varying from 2 to 5 minutes. When arterial blood was collected after this adaptation period of 5 minutes, the blood gas values did not differ from those obtained during the premask period.

Because of the fact that expiratory $\dot{V}$ recorded by the proximal pneumotachograph did not differ from the values recorded by the distal one, it was concluded that the expiratory $\dot{V}$was sufficiently laminar at the exit of the mask to allow accurate $\dot{V}$ measurement.

Upon increasing the dead space of the breathing apparatus progressively, significant modifications of some pulmonary and blood gas values were observed when the dead space was greater than 25% of the normal V_t (Table 1).

The signals recorded through the intrapleural needle and the balloon catheter were found to give a flat frequency response. However, the same pressure variation measured by the saline solution-filled catheter were 10 to 80% greater, according to the pump frequency (1 to 5 Hz). The in vivo comparison of the 3 different Ppl measuring methods are shown in Table 2. The pulmonary values obtained by each method were statistically compared, using the Student's *t* test for paired data. The modification in Ppl measurements induced by changing the position of the esophageal balloon catheter and the site of pleura puncturing are shown (Tables 3 and 4). The mean maxd Ppl of the different calves were greatest in the ventral site of the thorax and in the caudal thoracic portion of the esophagus, less in the dorsal site of the thorax and the middle thoracic portion of the esophagus, and least in the cranial site of the thorax and the cranial thoracic portion of the esophagus. However, the distribution was not identical in all the calves when considered individually.

In one instance, the Ppl measurements with the balloon catheter was disturbed because of the presence of air in the esophageal lumen. This was concluded from the fact that the catheter could be moved too easily and from the uncommon Ppl recordings. It disappeared quickly.

Discussion

From the results obtained it was concluded that, for accurate measurements of pulmonary functions in calves, the dead space of the breathing apparatus has to be smaller than 25% of the normal V_t. This is in good agreement with the observations of Kiorpes et al[5] who observed that placing a mask (with a dead space of 40% of the V_t) induced a decrease in Pa_{O_2}. This seems to be a problem only in calves. In persons, the dead space of the mouth piece is small, and in horses, a relatively high V_t is observed. The constant ptyalism is also a specific problem in calves. It justifies the necessity of the upper orientation of the airway opening of the mask (Fig 1). The in vitro comparison of the 3 Ppl measuring methods gives results similar to those described by Senterre and Geubelle,[12] using pediatric equipment. Indeed, in both studies, the frequency response of the saline solution-filled catheter was not flat to 5 Hz. There were no significant differences between the pulmonary function val-

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[j] Flow calibration set, Gould Godart, Bilthoven, The Netherlands.

ues measured with the balloon catheter, the tip of which was positioned 80 cm from the nares, and the intrapleural needle introduced into the right 9th intercostal space on a line running from the tuber coxae to the shoulder joint. These 2 positions being in the same horizontal plane of the thorax, it was postulated that, in nonanesthetized standing calves, the changes in esophageal pressure measured by the balloon catheter reflect local changes in pleural pressure. This is in good agreement with the conclusions reported for dogs,[13] persons,[14] and ponies.[15]

On the other hand, absolute Ppl values measured simultaneously by the intrapleural and esophageal methods were more homogenous in the present study than data reported for dogs[13] and ponies.[15] This could be due to the small elastance of the esophageal wall in calves (0.1 kPa/ml).[k] Indeed, the difference between the intrapleural and intraesophageal pressure measurements is proportional to the compliance of the manometric system and the elastance of the esophageal wall.[16] Therefore, the percentage of error committed in identifying the endoesophageal with intrapleural pressure changes, calculated from the equation proposed by Milic-Emili and Petit,[17] is around 0.5% for the equipment and animals described here.

This is negligible and smaller than in persons[12] and dogs.[16] The significant difference between the pressures recorded in the cranial and middle thoracic portions of the esophagus and the pressure gradient between the dorsal and ventral sites of the thorax (0.02 kPa/cm of descent) observed in the present study are in good agreement with data reported in other species.[15,18,19]

Nevertheless, the difference recorded in this study between the Ppl in the cranial and middle thoracic portion of the esophagus, these 2 sites being in the same horizontal plane of the thorax, and the difference between the dorsal and cranial thoracic positions, these 2 sites being also on the same horizontal level, suggest that pressure changes along the cephalocaudal dimensions would exist in standing unsedated cattle and would be due to a higher max Ppl in the caudal portion of the thorax (Tables 3 and 4). This finding also has been reported in dogs[18,19] and was attributed to nonuniform pulmonary ventilation and compliance. The proximity of the abdominal components could be another explanation of this higher max Ppl in the caudal portion of the thorax. It has been shown that the increase in the maxd Ppl observed after rumen inflation is due to a higher expiratory level of the intrapleural pressure.[20]

Necropsy examination of other calves having the same size as those described in the present study showed that when the catheter tip was positioned 5 cm caudodorsal of the trachea bifurcation, the balloon was positioned between the crossing point with the aorta and the 2 largest caudal mediastinal lymph nodes. Therefore the influence of the periesophageal structure, mainly the cardiovascular and lymphatic, on the endoesophageal pressure measurements seems to be minimum on this level. This could explain the smaller SD observed in the present study of the Ppl values recorded in the middle thoracic portion of the esophagus than in the 2 other esophageal positions.

It was concluded that if some technical and methodological requirements are respected, the pattern and positioning of the face mask and the balloon catheter proposed in this study allow making accurate $\dot{V}$, V_t, and Ppl measurements in nonanesthetized and unsedated standing calves.

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