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Effects of tracheal orientation on development of ventilator-associated pneumonia: an experimental study

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Abstract *Purpose:* Orientation of the trachea and tracheal tube below horizontal may prevent aspiration of oropharyngeal secretions into the lungs, which is a pivotal pathway in the pathogenesis of ventilator-associated pneumonia (VAP). The incidence of VAP was evaluated in swine with orientation of trachea and tracheal tube above horizontal (model of semirecumbent position, currently recommended in patients) and below horizontal. *Methods:* Twenty-six mini-pigs were randomized into four groups: (A) eight mechanically ventilated with orientation of trachea 45° above horizontal for 72 h. In the remaining groups (B, C, D) the trachea was oriented 10° below horizontal, with (B) six mechanically ventilated for 72 h, (C) six mechanically ventilated for 72 h with enteral feeding, and (D) six mechanically

ventilated for 168 h with enteral feeding. At the end of the study period, all pigs were sacrificed and the clinical diagnosis of VAP was microbiologically evaluated. No antibiotics were administered.

Results: All eight pigs kept oriented with the trachea 45° above horizontal developed VAP and respiratory failure ($\text{PaO}_2/\text{F}_i\text{O}_2 = 132 \pm 139$ mmHg) with a median of 5.5 pulmonary lobes out of 6 colonized with average colonization of 9.3×10^7 CFU/g. None of the 18 pigs kept oriented with the trachea below horizontal developed VAP; 16 had sterile lungs, while 2, ventilated for 7 days, developed a low level of colonization. *Conclusions:* Orientation of the trachea above horizontal was uniformly associated with VAP and respiratory failure; positioning the trachea below horizontal consistently prevented development of VAP.

Keywords Mechanical ventilation · Pneumonia · Ventilator-associated pneumonia · Semirecumbent position · Intubation · Postural drainage · Pulmonary · Pigs · Nosocomial infections · Respiration · Artificial/adverse effects · Intratracheal/adverse effects · Respiratory tract infections/prevention and control · Aspiration/prevention and control

Introduction

Ventilator-associated pneumonia is a frequent nosocomial infection with average incidence of 10–20% in intensive care unit patients undergoing mechanical ventilation [1]. The stomach is a potential reservoir of pathogenic bacteria, which can colonize the upper aerodigestive tract through gastric reflux. Elevation of the head of the bed to more than 30° above horizontal (semirecumbent position) is a recommended strategy to reduce gastric reflux and subsequent aspiration of colonized gastric contents [2, 3]. However, the efficacy of this strategy to prevent VAP remains controversial [4].

Long-term animal studies have been conducted to ascertain safety and effects of endotracheal tube (ETT) and tracheal orientation on development of VAP. In sheep mechanically ventilated for 72 h, the authors showed that orientation of the ETT/trachea 30° above horizontal (model of semirecumbent position) was invariably correlated with heavy colonization of the lungs and pneumonia. In contrast, when the ETT/trachea was oriented at or below horizontal, the sheep did not develop VAP and maintained excellent lung function [5]. Anatomical and physiological differences between sheep (ruminants and herbivores) and humans (omnivores) raised some concerns about translation of the results into clinical practice [6]. Therefore, the relationship between ETT and tracheal orientation in development of VAP was studied in a swine model, an omnivore with gastrointestinal physiology similar to humans [7].

Materials and methods

This study was approved and conducted at the National Institutes of Health Animal Research Laboratory, Bethesda, MD, USA.

A detailed version of the methods is available in the Electronic Supplementary Material.

Study groups

Twenty-six adult female Yucatan mini-pigs were randomized into four groups:

- (A) TracheaUp: Eight pigs were mechanically ventilated in prone position for 72 h with the ETT/trachea oriented 45° above horizontal. Two of eight pigs received enteral feeding through an orogastric tube.
- (B) TracheaDown72: Six pigs were mechanically ventilated in prone position for 72 h with the ETT/trachea oriented 10° below horizontal.
- (C) TracheaDown72F: Six pigs were mechanically ventilated in prone position for 72 h with the ETT/

trachea oriented 10° below horizontal. All six pigs received enteral feeding through an orogastric tube.

- (D) TracheaDown168F: Six pigs were mechanically ventilated in prone position for 168 h with the ETT/trachea oriented 10° below horizontal. All six pigs received enteral feeding through an orogastric tube.

Tracheal orientation

Figure 1a, b shows the swine positions used to obtain orientation of ETT/trachea above or below horizontal. Figure 1c shows a representative swine tracheal fluoroscopy, which highlights the relative orientation of the neck and extrathoracic and intrathoracic trachea. The extrathoracic trachea (inside the neck) proceeds substantially parallel to the ventral wall of the neck, while the intrathoracic portion turns backward towards the spine, creating an angle around 11°. In the TracheaUp group, to achieve an orientation of the ETT/trachea 45° above horizontal (model of semirecumbent position), the head of the bed was oriented 30° above horizontal and a foam pillow was placed beneath the head. In the TracheaDown72, TracheaDown72F, and TracheaDown168F groups, to achieve an orientation of the trachea of 10° below horizontal, the head of the bed was tilted 10° below horizontal.

Animal handling

Pigs were anesthetized, orotracheally intubated with an endotracheal tube using a high-volume low-pressure cuff (Mallinkrodt Hi-Lo), and mechanically ventilated with tidal volume of 6–8 ml/kg, respiratory rate of about 14 breaths/min (range 10–20 breaths/min), and 5 cmH₂O positive end-expiratory pressure (PEEP). Ventilation was adjusted to maintain arterial partial pressure of CO₂ (PaCO₂) around 40 mmHg. The endotracheal cuff pressure was maintained at 25–30 cmH₂O.

Endotracheal suctioning was performed every 6 h by means of an open tracheal suction system. No antibiotics were administered at least 1 month before as well as throughout the study. Chest X-rays were taken after animal preparation, when clinically indicated, and also before the autopsies. No bacteria were administered.

Microbiology and pathology

All pigs were sacrificed at the end of the study period, or after development of severe respiratory failure (defined as PaO₂/F_iO₂ <200 mmHg). The trachea and lungs were aseptically removed. Samples of lung tissue were

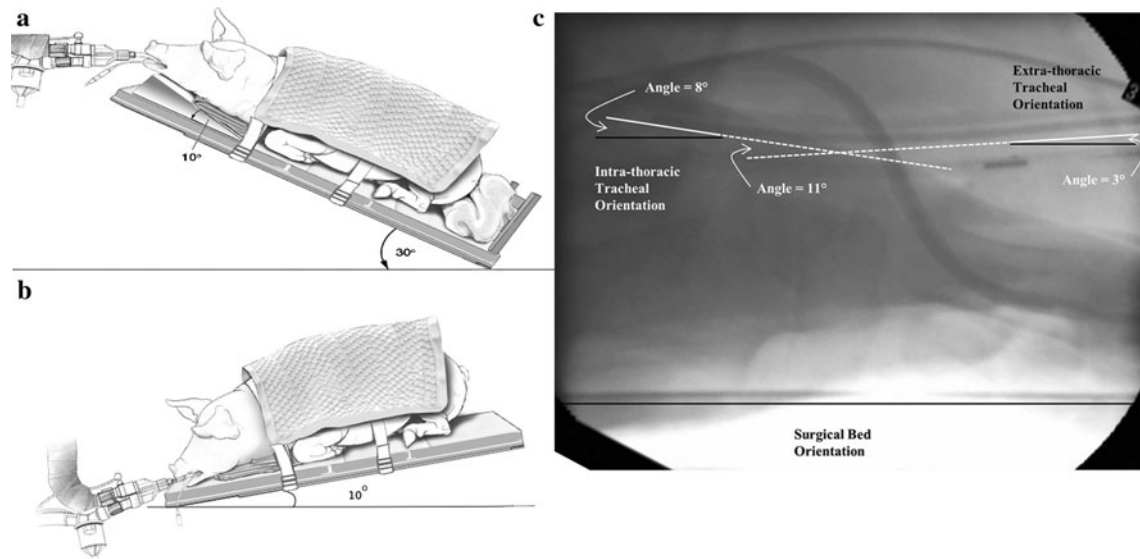


Fig. 1 Pig positioning. For pigs kept with orientation of the trachea 45° above horizontal, the bed was tilted 30° and a pillow was positioned below the head to obtain orientation of the trachea of 45° (a). For pigs kept with the trachea oriented 10° below horizontal the bed was tilted 10° (b). Pigs were not moved for the whole duration

collected from the inner parts of each of the six lobes and at the carina and 1–2 cm beyond the tip of the ETT for quantitative and qualitative microbiological analysis (see the Electronic Supplementary Material). Additional samples were collected from visibly abnormal areas of the lungs. Lung samples for histological analysis were obtained from four pigs from the TracheaUp group, ventilated for 72 h in semirecumbent position, to confirm the diagnosis of pneumonia.

Diagnosis of pneumonia

Pneumonia was defined as occurrence of new pulmonary opacities on chest X-ray associated with hypoxemia, presence of purulent tracheal secretions, gross findings suggestive of pneumonia at autopsy, and microbiological documentation of bacterial colonization.

Statistical methods

The two pigs in the TracheaUp group with enteral feeding were a separate group in the analysis in order to define the determinants of bacterial colonization (Fisher–Freeman–Halton test, exact logistic regression, Poisson regression) since presence/absence of enteral feeding had been independently associated with development of pneumonia, while in all tables and figures, as for the Karnaugh maps, they are grouped with the other six pigs ventilated with orientation of the trachea above horizontal. We made

of the study. c Representative fluoroscopy of mini-pig trachea and endotracheal tube taken in prone position; the extrathoracic portion of the tracheal tube is almost parallel to the horizontal (3° upward), while the intrathoracic portion is 8° downward

this choice because the two pigs with feeding in the head-up position behaved as the six pigs in the head-up position without feeding. For continuous measurement variables, the Kruskal–Wallis test was used and the Mann–Whitney test using the Bonferroni correction (Statistical, Electronic Supplementary Material).

Results

Study population

Twenty-six healthy Yucatan female mini-pigs (age 8 ± 2 months, weight 33 ± 6 kg) were studied (confirmed by clinical criteria and laboratory data).

Bacteriology

Table 1 and Fig. 2 summarize bacterial lung colonization (an extended version of this table is available in the Supplementary Appendix as Table 6). All pigs in the TracheaUp group, ventilated for 72 h with ETT/trachea oriented above horizontal, had heavily multibacterial colonized lungs (median of 5.5 lobes out of 6 and average colonization of 9.3×10^7 CFU/g with an average of three bacterial species each). All pigs in the TracheaDown72 and TracheaDown72F groups, ventilated for 72 h with ETT/trachea oriented below horizontal, had sterile lungs regardless of enteral feeding. Two of six pigs in the TracheaDown168F group, ventilated for 168 h with ETT/

Table 1 Bacterial lung colonization

Group	Pig	Right upper lobe	Right medium lobe	Right lower lobe	Left upper lobe	Left medium lobe	Left lower lobe
TracheaUp	5,620		CbS sp (1.40 × 10 ⁶)	CbS (5.20 × 10 ⁷)		CbS (3.50 × 10 ⁵), AeU (1.00 × 10 ⁵)	CbG (3.60 × 10 ³), CbS (3.10 × 10 ⁷), AeU (5.00 × 10 ⁶), CbS (2.80 × 10 ⁸)
TracheaUp	5,621	CbS (9.30 × 10 ⁷), CbG (8.30 × 10 ⁵)	CbS (2.70 × 10 ⁹)	CbS (1.90 × 10 ⁹)	CbS (1.50 × 10 ⁹)	CbS (1.40 × 10 ⁹)	SmM (2.20 × 10 ⁸), StU (4.70 × 10 ⁷), OkS (4.60 × 10 ⁶), StC (3.00 × 10 ⁴)
TracheaUp	5,639	SmM (8.40 × 10 ⁴), StU (3.70 × 10 ³)	SmM (4.40 × 10 ⁷), StU (4.00 × 10 ⁶)	SmM (1.30 × 10 ⁷), StU (1.20 × 10 ⁶)	SmM (5.00 × 10 ⁷), StU (2.10 × 10 ⁷)	SmM (8.90 × 10 ⁷), StU (3.10 × 10 ⁷)	SmM (2.20 × 10 ⁸), StU (4.70 × 10 ⁷)
TracheaUp	5,644	OkS (1.80 × 10 ⁸), StC (2.30 × 10 ⁶)	OkS (1.10 × 10 ⁹), StC (2.80 × 10 ³)	OkS (2.50 × 10 ⁸), StC (5.60 × 10 ⁶)	OkS (1.40 × 10 ⁷), StC (1.10 × 10 ⁵)	OkS (1.10 × 10 ⁹), StC (1.30 × 10 ⁷)	OkS (4.60 × 10 ⁶), StC (3.00 × 10 ⁴)
TracheaUp	5,652	EsC (1.70 × 10 ³), CbS (1.10 × 10 ⁹)	EsC (3.20 × 10 ³), ArP (2.00 × 10 ⁷)	EsC (1.20 × 10 ³), CbS (7.20 × 10 ⁸)	ShE (2.30 × 10 ³), CbS (7.10 × 10 ⁶)	ArP (4.20 × 10 ⁷), CbS (5.90 × 10 ⁷)	CbS (7.70 × 10 ⁷), AcS (4.70 × 10 ⁶)
TracheaUp	5,661	AcS (5.60 × 10 ⁷), BrV (5.90 × 10 ⁷)	CbS (5.30 × 10 ⁷), BrV (1.20 × 10 ⁶)	AcS (8.00 × 10 ⁷), BrV (8.70 × 10 ⁷)	AcS (1.00 × 10 ⁶), BrV (1.00 × 10 ³)	BrV (3.20 × 10 ³), StI (1.10 × 10 ³)	AcS (4.70 × 10 ⁶), StIP (4.90 × 10 ³), ArH (1.20 × 10 ⁴), OkS (4.10 × 10 ⁴)
TracheaUp	8,057	StIP (3.70 × 10 ⁷), ViV (3.00 × 10 ⁷)	Stm (5.00 × 10 ⁶)	StIP (5.60 × 10 ⁷)	StIP (1.00 × 10 ³)	StIP (2.20 × 10 ³)	OkS (4.10 × 10 ⁴)
TracheaUp	8,071	PsA (7.30 × 10 ³), StA (1.50 × 10 ⁴)	PsA (2.90 × 10 ⁶), StA (5.30 × 10 ⁵)	PsA (2.00 × 10 ⁶), StA (1.70 × 10 ⁶)	PsA (3.30 × 10 ⁴), StA (3.80 × 10 ⁶)	PsA (1.20 × 10 ⁶), StA (2.90 × 10 ⁶)	PsA (4.20 × 10 ⁶), StA (5.80 × 10 ⁶)
TracheaDown72	All six pigs	ArP (1.30 × 10 ⁶), MiL (5.10 × 10 ⁴)	ArP (4.00 × 10 ⁷), MiL (5.30 × 10 ⁵)	ArP (6.20 × 10 ⁷), MiL (2.80 × 10 ⁶)	ArP (6.40 × 10 ⁶), MiL (2.70 × 10 ⁵)	ArP (1.10 × 10 ⁷), MiL (1.00 × 10 ⁶)	ArP (8.80 × 10 ⁷), MiL (1.10 × 10 ⁶)
TracheaDown72F	All six pigs						
TracheaDown168F	Four pigs						
TracheaDown168F	725	Lis (8.70 × 10 ⁵)	StU (2.30 × 10 ⁴), EnF (5.60 × 10 ³)				
TracheaDown168F	723						Lis (4.00 × 10 ⁴)

Table summarizes lung bacterial colonization expressed as CFU/g in each lobe. Empty cells represent sterile lobes

AcS *Acinetobacter* sp., AeU *Aerococcus urinae*, ArH *Arcanobacterium haemolyticum*, ArP *Arcanobacterium pyogenes*, BrV *Brevundimonas vesicularis*, CbG *Corynebacterium genitalium*, CbS *Corynebacterium* sp., EnF *Enterococcus faecalis*, EsC *Escherichia coli*, Lis *Listeria* sp., MiL *Micrococcus luteus*, OkS *Oerskovia* sp., PsA *Pseudomonas aeruginosa*, ShE *Staphylococcus epidermidis*, SmM *Stomatococcus mucilaginosus*, StA *Streptococcus agalactiae*, StC *Streptococcus cristatus*, StI *Streptococcus intermedius*, StIP *Streptococcus porcinus*, Stm *Streptococcus sanguinis*, StU *Streptococcus suis*, ViV *Vibrio vulnificus*

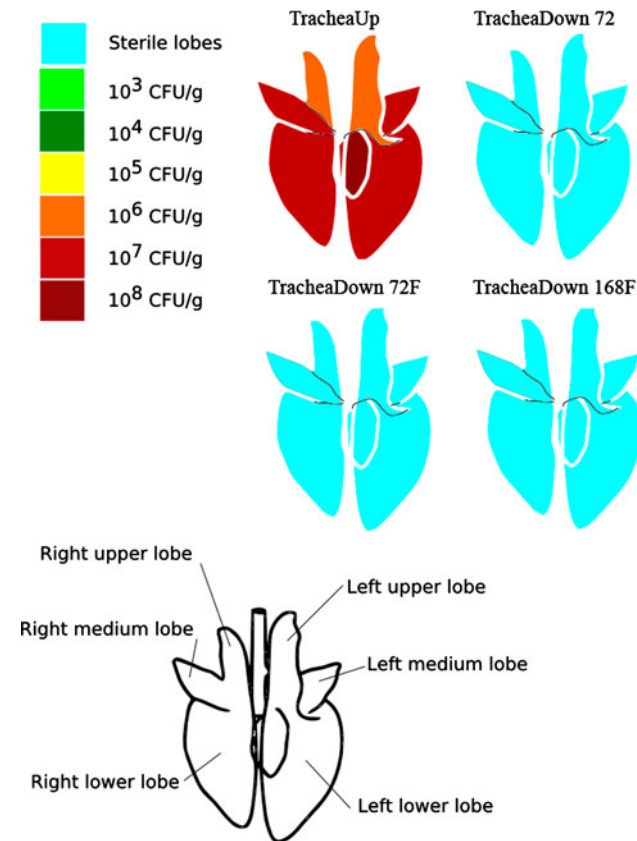


Fig. 2 Median of lung colonization (colony forming units/gram) in each lobe, group by group. The accessory lobe was considered part of the left lower lobe

trachea oriented below horizontal with feeding, experienced a low level of colonization.

The Fisher–Freeman–Halton test showed a significant association between test group and colonization in all trachea sample locations except in the region of the endotracheal tube tip. Logistic regression showed that the only significant predictor of bacterial colonization was the orientation of the ETT/trachea ($p = 0.0001$); the Poisson regression showed that the only significant predictor of number of lobes with bacteria colonization was the orientation of the ETT/trachea ($p < 0.0001$).

Correlation of bacteria presence in the lungs and in the trachea

We found a significant correlation between bacteria in lung lobes and bacteria at the level of ETT tip and carina in the TracheaUp group (Spearman's correlation, $r = 0.789$, $p < 0.0001$, see Electronic Supplementary Material). No significant correlation was found between the bacteria found in the mouth and stomach, and the bacteria found in the lungs (Tables 7 and 8 in Electronic Supplementary Material).

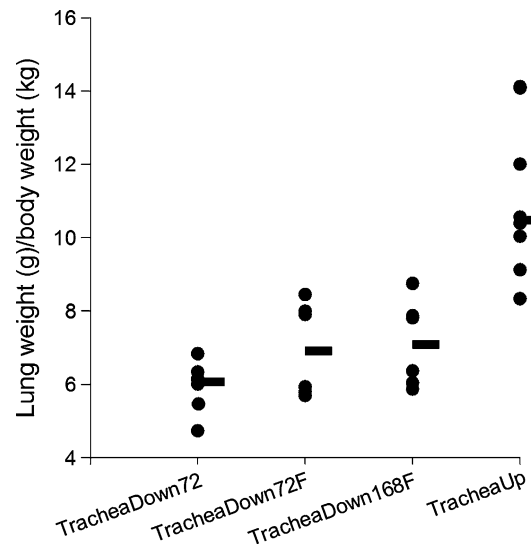


Fig. 3 Lung weight (g), measured at autopsy, normalized by body weight (kg). Median is reported as horizontal mark. Lung weights were compared among the four groups using the Kruskal–Wallis test. The group kept in semirecumbent position was statistically different from the other three groups ($p < 0.001$). The three groups kept with the trachea oriented 10° below horizontal were not different from each other

Lung pathology

When the chest was opened for autopsy lungs in the TracheaUp group, the lungs appeared purple, hard, and congested as shown in Fig. 1a, e of the Electronic Supplementary Material; the lungs of a pig ventilated with the ETT/trachea oriented below horizontal are shown for comparison. Purulent mucus was found inside the bronchi of all pigs in the TracheaUp group (Fig. 1c, Electronic Supplementary Material). Lung weight normalized by body weight of pigs in the TracheaUp group was nearly double compared with pigs ventilated with the ETT/trachea oriented below horizontal, suggesting the presence of marked lung edema ($p < 0.0001$, Wilcoxon test) (Fig. 3).

Histological analysis of pigs in the TracheaUp group consistently confirmed grossly abnormal lungs and the presence of severe diffuse bronchopneumonia. Lungs were consistently described as with “suppurative bronchopneumonia with intralesional bacteria and peribronchiolar and perivascular lymphocytic infiltrates” (Fig. 5d, Electronic Supplementary Material). No pigs in the TracheaDown72, TracheaDown72F or TracheaDown168F groups, ventilated with the ETT/trachea oriented below horizontal, met any criteria for pneumonia, and the lungs appeared naturally inflated at autopsy (Fig. 5e, Electronic Supplementary Material).

Clinical course

All 18 pigs with the ETT/trachea oriented below horizontal (TracheaDown72, TracheaDown72F, and

TracheaDown168F groups) completed the study (respectively, 72 or 168 h of mechanical ventilation). Two of eight pigs from the TracheaUp group completed 72 h of mechanical ventilation. The remaining six pigs from the TracheaUp group did not complete the study due to development of severe respiratory failure (see below); the average length of mechanical ventilation in the TracheaUp group was 62 ± 9 h.

All eight pigs from the TracheaUp group developed severe respiratory failure with serious hypoxemia ($\text{PaO}_2/\text{F}_i\text{O}_2 = 132 \pm 139$ mmHg) and 40% reduction of respiratory system compliance (Table 2; Fig. 4; Table 3 and 4 Electronic Supplementary Material); no pigs ventilated with the ETT/trachea oriented below horizontal (TracheaDown72, TracheaDown72F, and TracheaDown168F groups) developed any degree of respiratory failure or opacities on chest X-ray (Fig. 5b, Electronic Supplementary Material). There was no evidence of left ventricular failure (pulmonary balloon occlusion pressure recorded on five pigs was 5 ± 4 mmHg). Upon development of respiratory failure, minute volume was

increased by 50% to maintain arterial pH and PCO_2 near constant. In contrast, all pigs ventilated with the ETT/trachea oriented below horizontal (TracheaDown72, TracheaDown72F, and TracheaDown168F groups) maintained excellent lung function throughout the entire study period (Table 2) (Table 3 and 4, Electronic Supplementary Material). Surprisingly, there was not an analogous increase in white blood cells in the TracheaUp group (increase of $6.6 \pm 6.6/10^3/\text{ml}$ for TracheaDown168F, $0.2 \pm 3.4/10^3/\text{ml}$ for TracheaDown72F, $5.2 \pm 1.5/10^3/\text{ml}$ for TracheaDown72, and $1.0 \pm 3.3/10^3/\text{ml}$ for TracheaUp, $p = 0.03$) (Table 9, Electronic Supplementary Material).

Hemodynamics

As shown in Table 2 (also see Tables 4 and 5, Electronic Supplementary Material), ventilation with the ETT/trachea oriented below horizontal was not associated with respiratory mechanics or hemodynamic impairment. In

Table 2 Physiological variables

	TracheaDown168F	TracheaDown72F	TracheaDown72	TracheaUp	<i>p</i> value
pH					
Start of experiment	7.466 ± 0.029	7.508 ± 0.036	7.488 ± 0.069	7.497 ± 0.041	$p = 0.17$
End of experiment	7.502 ± 0.016	7.554 ± 0.018	7.520 ± 0.042	7.438 ± 0.113	
Delta (end–start)	0.036 ± 0.025	0.045 ± 0.044	0.031 ± 0.066	-0.059 ± 0.122	
$\text{PaO}_2/\text{F}_i\text{O}_2$					
Start of experiment	573 ± 110	511 ± 23	565 ± 50	530 ± 42	$p < 0.01$
End of experiment	452 ± 61	488 ± 16	496 ± 28	132 ± 139	
Delta (end–start)	$-121 \pm 100^*$	$-24 \pm 33^*$	$-69 \pm 53^*$	-398 ± 158	
PaCO_2					
Start of experiment	40 ± 3	41 ± 4	40 ± 6	40 ± 4	$p = 0.10$
End of experiment	41 ± 3	38 ± 2	41 ± 3	52 ± 17	
Delta (end–start)	1 ± 5	-2 ± 4	1 ± 5	12 ± 15	
Respiratory rate (breaths/min)					
Start of experiment	13 ± 1	16 ± 3	13 ± 2	15 ± 2	$p < 0.01$
End of experiment	13 ± 2	15 ± 2	14 ± 2	29 ± 16	
Delta (end–start)	$0 \pm 2^*$	$-1 \pm 2^*$	$0 \pm 2^*$	15 ± 15	
Plateau pressure (cmH₂O)					
Start of experiment	18 ± 2	16 ± 1	16 ± 2	16 ± 1	$p = 0.05$
End of experiment	20 ± 2	17 ± 2	18 ± 2	23 ± 5	
Delta (end–start)	1 ± 2	1 ± 2	2 ± 1	7 ± 6	
CPL (respiratory system) (L/cmH₂O)					
Start of experiment	25 ± 2	23 ± 4	25 ± 3	24 ± 5	$p = 0.03$
End of experiment	22 ± 3	21 ± 5	20 ± 3	14 ± 5	
Delta (end–start)	$-4 \pm 3^*$	$-2 \pm 3^*$	$-4 \pm 2^*$	-10 ± 8	
Mean arterial pressure (mmHg)					
Start of experiment	108 ± 17	120 ± 23	95 ± 12	101 ± 27	$p = 0.53$
End of experiment	97 ± 19	123 ± 18	94 ± 13	99 ± 27	
Delta (end–start)	-11 ± 13	3 ± 15	-1 ± 23	-2 ± 27	

Start of experiment refers to blood gas data collected 6 h after experimental orientation of the trachea was obtained. End of experiment refers to the last set of data collected, after the scheduled time (72 or 168 h) or immediately before sacrifice. Respiratory system compliance was calculated as tidal volume/(plateau pressure – PEEP)

$\text{PaO}_2/\text{F}_i\text{O}_2$ arterial partial pressure of oxygen/inspired oxygen fraction, CPL respiratory system compliance

Changes occurred across the experimental period were compared between groups with the Kruskal–Wallis test; for multiple comparison, Wilcoxon test was used, correcting the *p* value for the number of multiple comparisons performed. * $p < 0.05$ versus TracheaUp

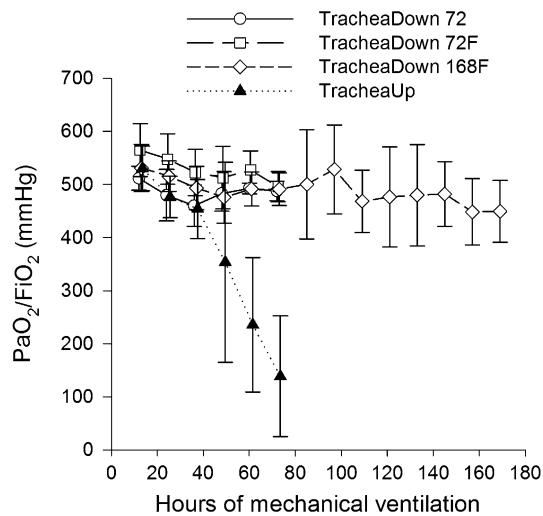


Fig. 4 $\text{PaO}_2/\text{F}_i\text{O}_2$ as a function of time in the four study group; data are presented as mean \pm standard deviation (SD). Only two pigs in the semirecumbent group completed the 72 h of study; one was sacrificed after 48 h, two after 54 h, one after 60 h, and two after 66 h. We calculated the rate of $\text{PaO}_2/\text{F}_i\text{O}_2$ change (slope) over time from 12 to 72 h, ignoring the remaining time of the TracheaDown168F (trachea oriented 10° below horizontal 168 h group) and tested for slope equality among the four groups using the Kruskal–Wallis test. Since the overall test was significant ($p < 0.001$) we performed pairwise comparisons. The group kept in the semirecumbent position was statistically different from the other three groups ($p < 0.001$). The three groups of pigs kept with the trachea oriented 10° below horizontal were not different from each other

two pigs of the TracheaUp group, inotropic support to maintain mean arterial pressure above 65 mmHg was necessary at the end of the study.

Enteral nutrition and regurgitation

Regurgitation was recorded in 3 of 12 pigs ventilated with the ETT/trachea below horizontal (TracheaDown72F and TracheaDown168F groups). In those animals, infusion of enteral feeding was halved, but never stopped.

Discussion

In swine mechanically ventilated up to 7 days without antibiotic administration, orientation of ETT/trachea above or below horizontal was the main determinant of lung infection and development of VAP.

Literature has highlighted two possible sources of bacteria which can reach and colonize the lungs: oro-tracheal flora and gastric flora [8]. When the human patient is placed in semirecumbent position, the esophagus and extrathoracic trachea are almost parallel to the bed (elevated to 45°), while the intrathoracic trachea is

approximately 20° more vertical. Such positioning invariably reduces gastric reflux [9]; however, pooling of oropharyngeal secretions above the endotracheal tube cuff may enhance subsequent leakage along the longitudinal folds of the cuff, leading to possible lung colonization by oropharyngeal flora [10]. Conversely, in supine position, the esophagus and extrathoracic trachea are almost horizontal, while the intrathoracic trachea is oriented 20° towards the back. This orientation may theoretically reduce the driving pressure of secretions across the cuff, but it is invariably associated with an increase in gastric reflux and possible aspiration of gastric content [9]. The respective role of pulmonary aspiration of gastric and oropharyngeal contents in the development of VAP has been extensively studied in human patients without conclusive results [3, 4, 8, 9, 11]. Interestingly, possible advantages of both semirecumbent and supine positions are based on enhanced gravitational drainage of secretions. Theoretically, orientation of the ETT/trachea below horizontal should allow clearance of all oropharyngeal, gastric contents and intratracheal [12] secretions through the mouth and ultimately avoid pulmonary aspiration and help prevent VAP. Conversely, orientation of the ETT/trachea above horizontal will likely result in movement of secretions across the ETT cuff and towards the lungs. It is important to emphasize that the safest position for an unconscious patient (without protection of the respiratory tract from aspiration) is lateral with some degree of Trendelenburg to assure the flow of gastric material toward the mouth and not into the trachea [13].

Extrathoracic trachea orientation is similar in humans and swine, while the intrathoracic trachea in swine has a lower inclination towards the back (10°). The extrathoracic trachea has the same orientation in supine and prone position, while the intrathoracic trachea in supine position is tilted downwards (facilitating fluid movement towards the lungs) and in prone position is oriented upwards (preventing fluid movement towards the lungs). Swine in this study were positioned prone, hence the body position was favorable for prevention of VAP [14]. An anatomical difference between human and swine is the alignment of the oral cavity; in swine, the oral cavity is directly aligned with the pharynx and the tracheal opening. Hence, the orientation of the body in swine (supine versus prone) does not affect secretion pooling in the retropharynx and drainage into the mouth. Use of prone position is not believed to be a factor influencing the results since tracheal orientation varied above and below horizontal in the four study groups. Some animal data from control experiments, not included in the present analysis, support this speculation. Two pigs were mechanically ventilated in supine position for 72 h, with the head of the bed oriented 10° below horizontal (as in TracheaDown72, TracheaDown72F, and TracheaDown168F groups) and received enteral feeding. Both pigs did not develop pneumonia and showed sterile lungs at autopsy. One

additional pig was ventilated supine for 72 h with orientation of trachea 45° above horizontal (as in the TracheaUp group) for a radiological study. This pig developed clinical pneumonia (radiologically confirmed, Fig. 6, Electronic Supplementary Material).

This long-term animal study aimed to model some of the determinants of VAP: pigs were deeply sedated, intubated, and mechanically ventilated. All of these conditions are known risk factors for VAP. The test groups were designed to primarily explore the efficacy of two different tracheal orientations, hence body orientation, in the prevention of VAP; and secondarily, how other known risk factors for VAP [15], such as enteral nutrition and length of mechanical ventilation, could affect such results. Importantly, only orientation of the ETT/trachea above horizontal appeared to be a major determinant of lung colonization and pneumonia. Since enteral nutrition [3, 16] and length of mechanical ventilation are known risk factors for VAP, their relative effect was evaluated only in the TracheaDown72, TracheaDown72F, and TracheaDown168F groups, where the pigs were ventilated with the ETT/trachea oriented below horizontal.

The incidence of VAP in this study is higher (100% of swine in the TracheaUp group developed VAP during 72 h of mechanical ventilation) than observed in human patients, where it ranges from 5% to 20% [1, 17]. The rate of pneumonia observed in our study is consistent with all published animal models of prolonged mechanical ventilation and suggests that this model, without requiring injection of extrinsic pathogens, is solid and reproducible. An animal model, in which the incidence of the studied disease is 100%, seems ideal to evaluate the efficacy of possible prevention strategies. Early studies by Johanson on mechanically ventilated baboons, without use of antibiotics, showed that Gram-negative bacillary pneumonia developed in all baboons [18], while when antibiotic prophylaxis was administered, all baboons become colonized, but pneumonia developed in only 16% of the baboons [19]. Likewise, Marquette et al. [20] developed a model of endogenously acquired pneumonia as a result of prolonged mechanical ventilation. Incidence of pneumonia, with and without antibiotic prophylaxis, was 94% and 44%, respectively. Previous results in sheep [5, 12] consistently found higher incidences of pneumonia when the ETT/trachea was oriented above horizontal. Several possible reasons could explain the higher incidence of VAP in this study's animal model in comparison with the human: First, most, if not all, patients during their clinical history receive some antibiotics, prophylactic or therapeutic, which greatly reduces the burden of endogenous oropharyngeal pathogens [21]. Secondly, not all patients

present pathogens in the oropharynx at admission, and the degree of oral and pharyngeal colonization at admission has been strongly related with the odds for development of pneumonia [22], while the upper airways of swine may carry bacteria with a higher degree of virulence. We could not highlight the routes of colonization in our experimental model since an extremely weak correlation was identified between bacteria found in the mouth and in the stomach, and bacteria found in the lungs. Only 25% of the 24 different bacteria that were found in the lungs were also present in the mouth or stomach (Tables 7 and 8, Electronic Supplementary Material). Two or three bacteria from the mouth were sampled, but it is known that the mouth hosts hundreds of bacteria. Thus, bacteria able to produce disease could have been present in low concentration. It may also be speculated that the test samples may not be representative of the posterior oropharynx and that more frequent sampling could have highlighted the route of colonization.

This study aimed to clarify a physiological mechanism, but also a possible clinical translation (accounting for the anatomical differences between swine and humans) may be positioning the human patient on one side with the head of the bed slightly tilted below horizontal (lateral-Trendelenburg). A pilot, randomized clinical trial study conducted at the Massachusetts General Hospital showed the feasibility of such body position [23] and its efficacy in increasing the number of ventilator-free days. Similarly, a study on newborns showed that lateral-horizontal orientation of the endotracheal tube is associated with a reduced rate of ventilator-associated pneumonia [24]. Moreover, continuous lateral rotational therapy has been used without major complications [25]. A randomized trial comparing semirecumbent position and lateral-Trendelenburg position in intubated and ventilated patients is currently ongoing (www.clinicaltrials.gov number: NCT01138540).

Conclusions

This study suggests that ventilation with the ETT/trachea oriented below horizontal may be superior to the semi-recumbent position in preventing VAP, by promoting outward drainage of bacteria-laden oropharyngeal secretions and avoiding bacterial translocation from the oropharynx into the lungs.

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