Public FDA Workshop

Current State and Further Development of Animal Models of Serious Infections Caused by Acinetobacter baumannii and Pseudomonas aeruginosa

March 1, 2017

Ventilated Pig Models of *Pseudomonas aeruginosa* Pneumonia

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Presenter Disclosure Information

Gianluigi Li Bassi, MD, PhD

Compensated service for a relevant commercial entity

1.	Hill-Rom	Research Funds
2.	Bayer	Research Funds, Honoraria for Lectures
3.	Covidien	Research Funds, Honoraria for Lectures
4.	Ciel Medical	Research Funds
5.	Theravance	Research Funds
6.	Medimmune	Research Funds
7.	Cubist	Research Funds
8.	Cardeas Pharma	Research Funds
9.	Lunguard	Research Funds
10.	Biovo Technologies	Research Funds
11.	Toray Ltd	Research Funds

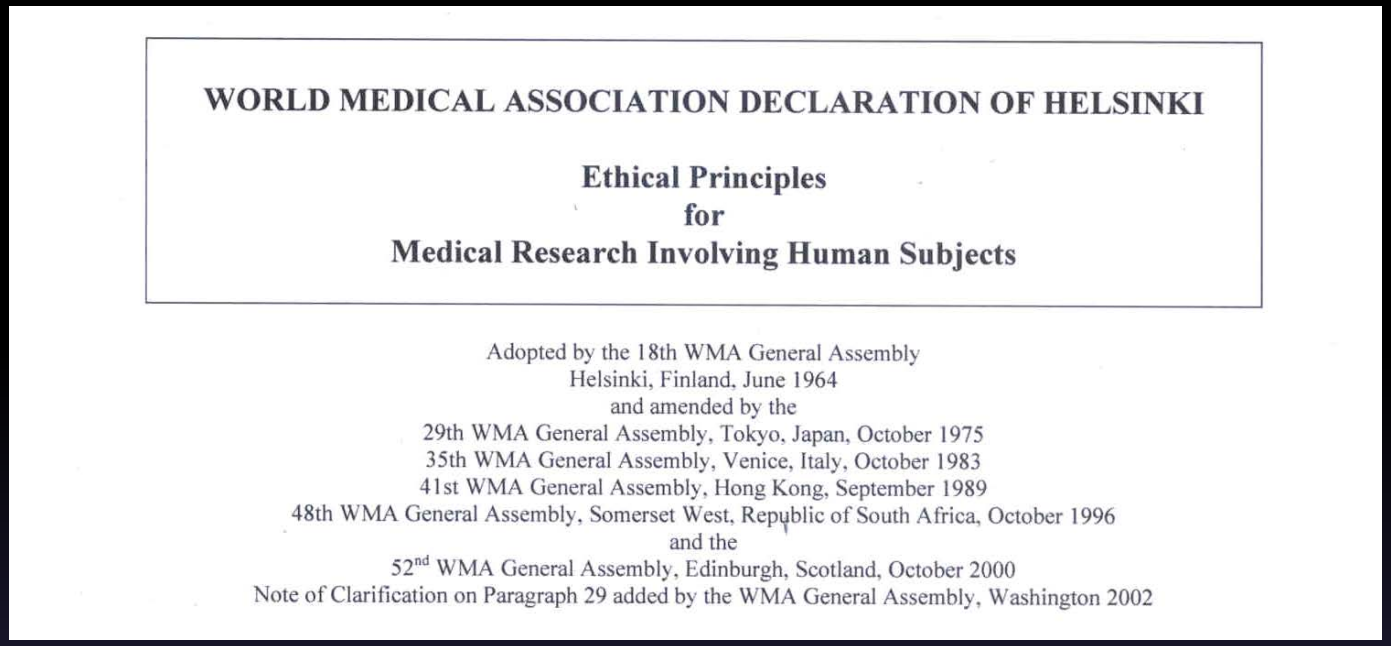
Royalties

1.	NIH	Development of the Mucus Slurper

Presenter will deliver content that is evidence-based and conforms to the generally accepted formats of scientific data presentations. Any and all clinical recommendations made relating to patient care will be based on the best available scientific evidence and not biased by any commercial entity's interest.

Presenter will use scientific or generic names in referring to products in their lectures or enduring materials. *For any product that will be mention, both the benefits and limitations of that product will be discussed and those of the products in that class of drugs.* Presenters will not stress one product over another without scientific evidence for recommendations.

BACKGROUND AND RATIONALE FOR THE USE OF LARGE ANIMAL MODELS OF P.AERUGINOSA PNEUMONIA



WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles
for
Medical Research Involving Human Subjects

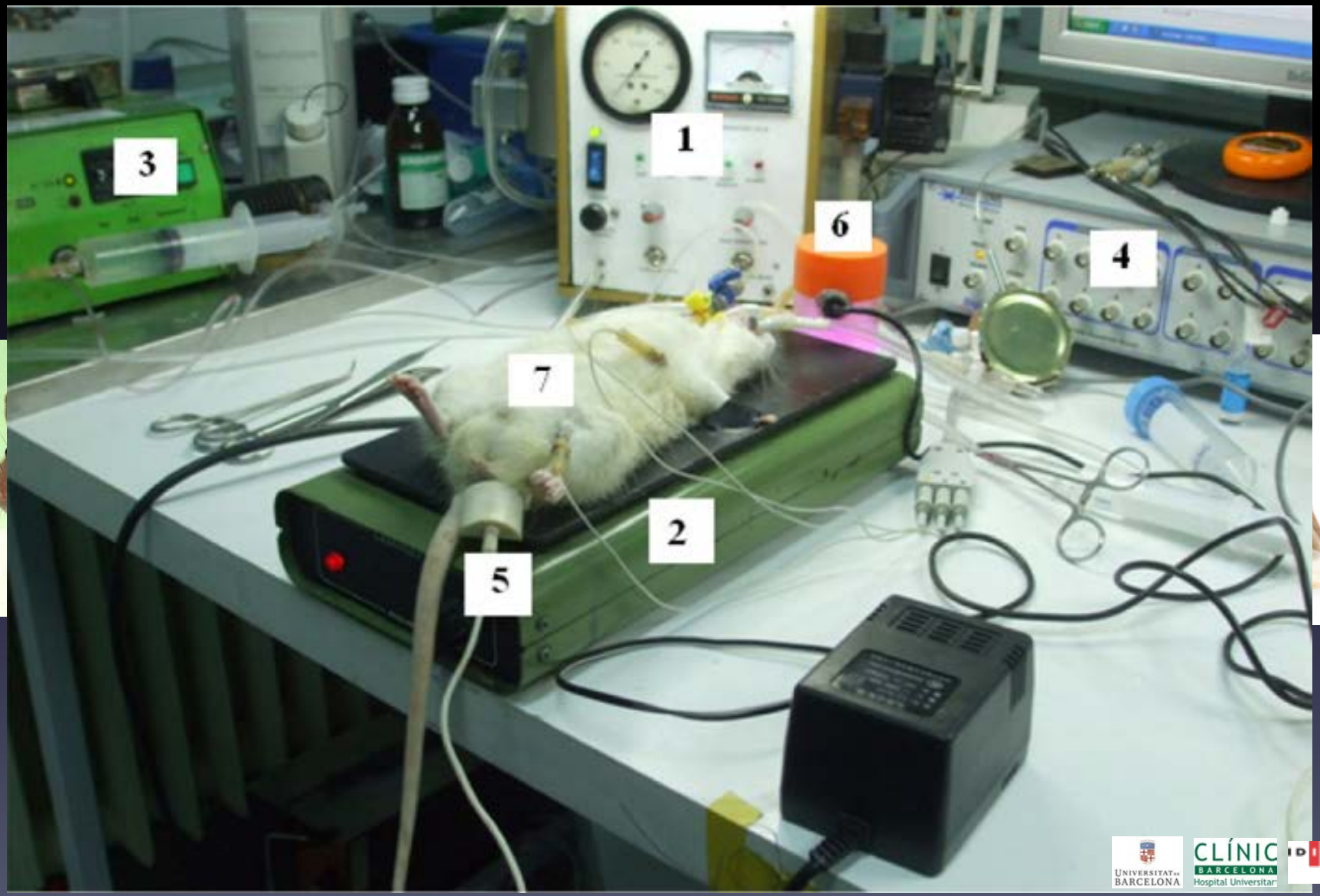
Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002

"Clinical research must conform to the moral and scientific principles that justify medical research and **should be based on laboratory and animal experiments or other scientifically established facts.**"

Rationale for the use of pneumonia animal models

- To elucidate pathogenesis of the disease (in the absence of confounding factors)
- To characterize the role of bacterial and host factors in the disease
- To test efficacy and safety of novel preventive strategies and therapies

Small Animal Models of *P.Aeruginosa* Pneumonia



P. aeruginosa Pneumonia Models in Small Animals

Morphologic and Microbiologic Features of P.aeruginosa Pneumonia in normal Hamsters. Coalson JJ, Higuchi JH, Williams ML, Johanson
Exp and Mol Pathology 1986, 45: 193-206

Effects of parenterally administered ciprofloxacin in a murine model of pulmonary Pseudomonas aeruginosa infection mimicking ventilator-associated pneumonia. Kaneko Y, Yanagihara K, Kuroki M, Ohi H, et al. *Chemotherapy*. 2001 Dec;47(6):421-9.

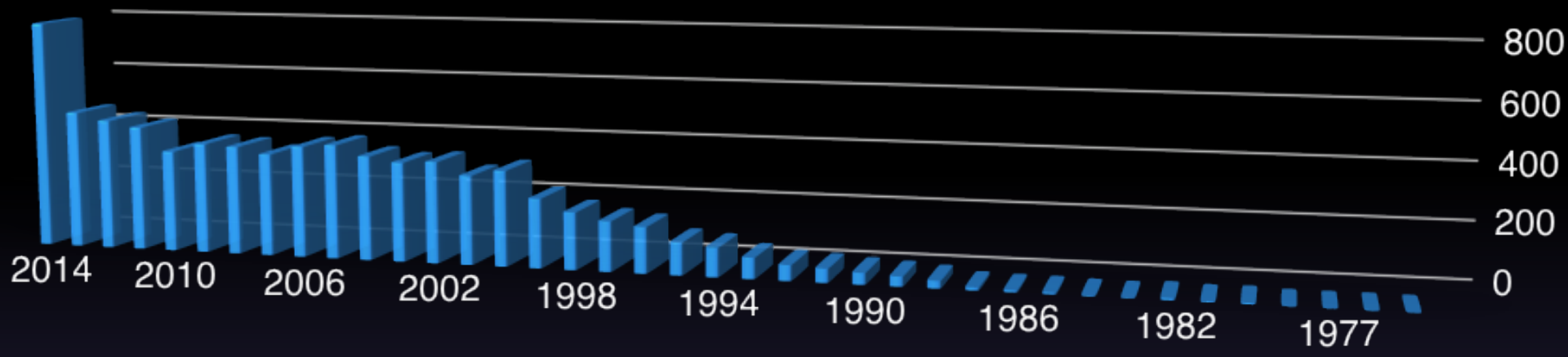
Efficacy of colistin combination therapy in a mouse model of pneumonia caused by multidrug-resistant Pseudomonas aeruginosa. Aoki N, Tateda K, Kikuchi Y, et al. *J Antimicrob Chemother*. 2009 Mar;63(3):534-42. Epub 2009 Jan 14.

In vivo efficacy and *pharmacokinetics of biapenem in a murine model of ventilator-associated pneumonia with Pseudomonas aeruginosa.* *Yamada K, Yamamoto Y, Yanagihara K, et al.* J Infect *Chemother*. 2012 Aug;18(4):472-8. Epub 2012 Jan 5.

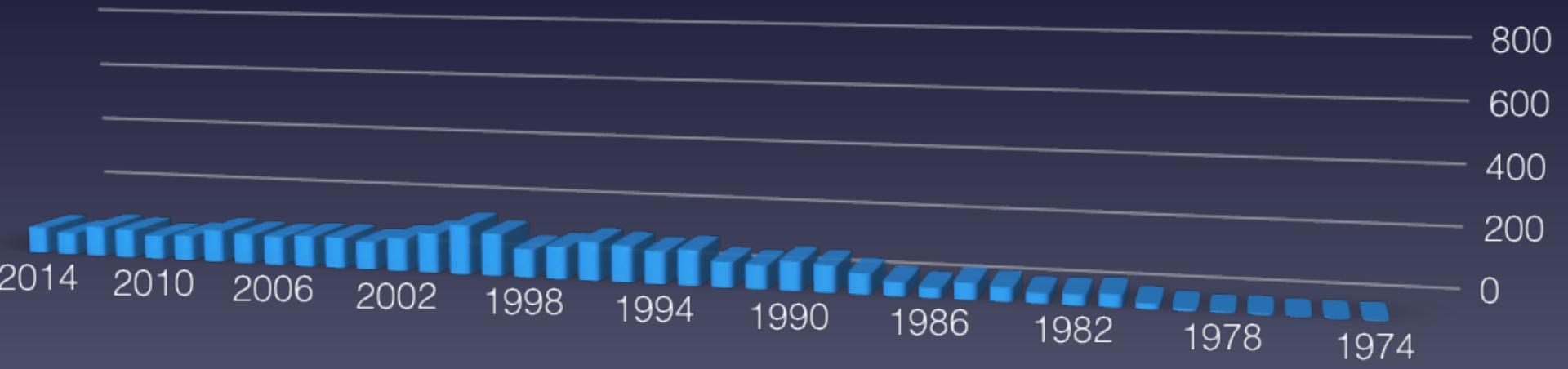
New model of ventilator-associated pneumonia in immunocompetent rabbits. Charles PE, Piroth L, Desbiolles N, et al. *Crit Care Med.* 2002 Oct;30(10):2278-83.

Candida albicans impairs macrophage function and facilitates Pseudomonas aeruginosa pneumonia in rat. Roux D, Gaudry S, Dreyfuss D, et al. *Crit Care Med*. 2009 Mar;37(3):1062-7.

1974-2014 Original Articles on pneumonia in Mice, Rats and Rabbits



1974-2014 Original Articles on pneumonia in Pigs, Sheep, Dogs and Baboons



Source: MEDLINE

Mouse Models of *P.aeruginosa* Pneumonia: BENEFITS

- Mouse:
  - Smallest mammal
  - Rapid reproductive rate

  - Genetic and physiological similarities to humans
  - Potentials for extensive genome manipulations
  - Unique strains and genetically engineered mutants
- Limited costs

Mouse Models of *P.aeruginosa* Pneumonia: LIMITATIONS

Table 2. *Select differences between mice and humans relevant to pneumonia*

Feature	Mice Differ From Humans In:
Anatomy of respiratory tract	1. Proportionally larger nasal surface area 2. Fewer, less symmetric airway branches 3. No respiratory bronchioles 4. Few mucous or serous cells 5. No submucosal glands below trachea
Physiology of respiratory tract	1. Obligate nose breathers 2. Weak cough reflex
Pattern recognition receptors	1. Cellular expression of TLRs 3, 5, and 9 2. Ligand specificity of TLRs 2, 4, and 9 3. Ligand specificity of Nod1
Antimicrobial secretions	1. Pattern of lysozyme secretion 2. Absence of hypothiocyanite
Neutrophils	1. Lower circulating counts 2. No α-defensins

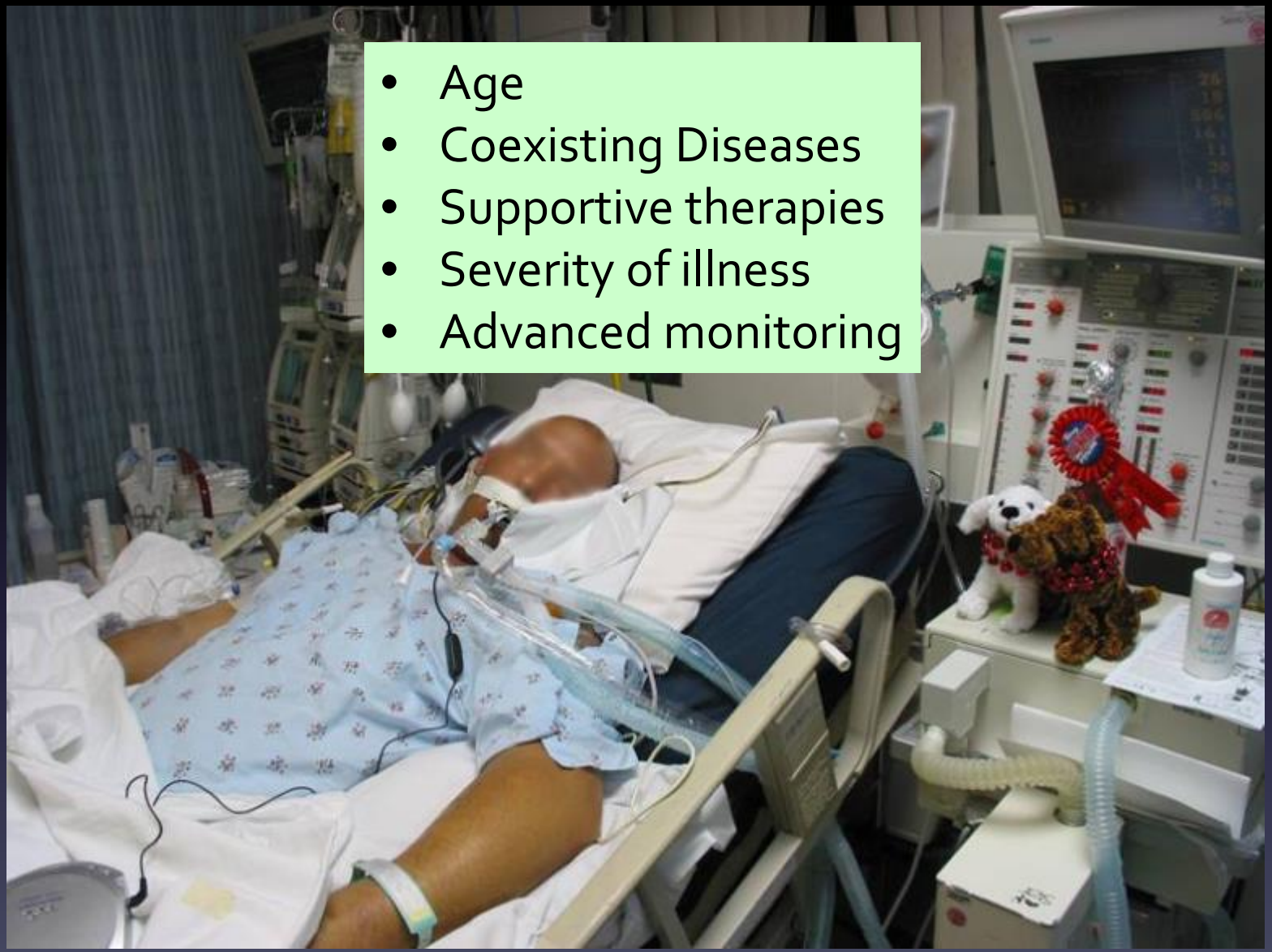
TLRs, Toll-like receptors; Nod1, human nucleotide binding and oligomerization domain protein 1.

Am J Physiol Lung Cell Mol Physiol 294: L387–L398, 2008.

Small Animal Models of *P.aeruginosa* Pneumonia:

OTHER LIMITATIONS

- Long term mechanical ventilation is unfeasible
- Limited ability to provide critical care to severely ill animals in septic shock
- As for VAP, primary pathogenic mechanism, through aspiration of oropharyngeal pathogens is difficult to reproduce
- The ability of experiments in small animals to predict the long-term effectiveness and safety of therapies in humans remains controversial
- ICU-related concomitant conditions difficult to replicate


• Age
• Coexisting Diseases
• Supportive therapies
• Severity of illness
• Advanced monitoring

CHEST

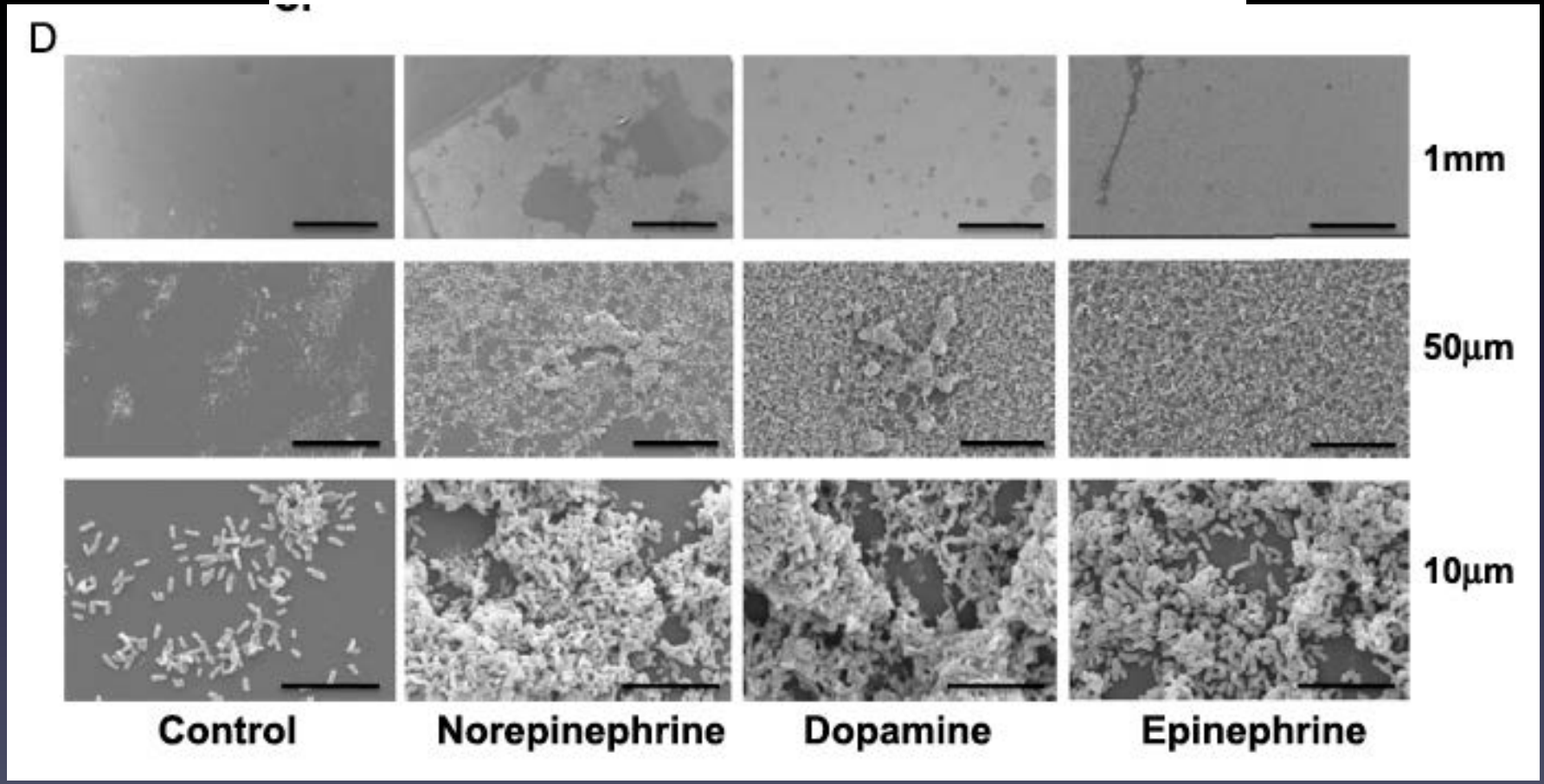
Original Research

CRITICAL CARE

Pseudomonas aeruginosa-Catecholamine Inotrope Interactions

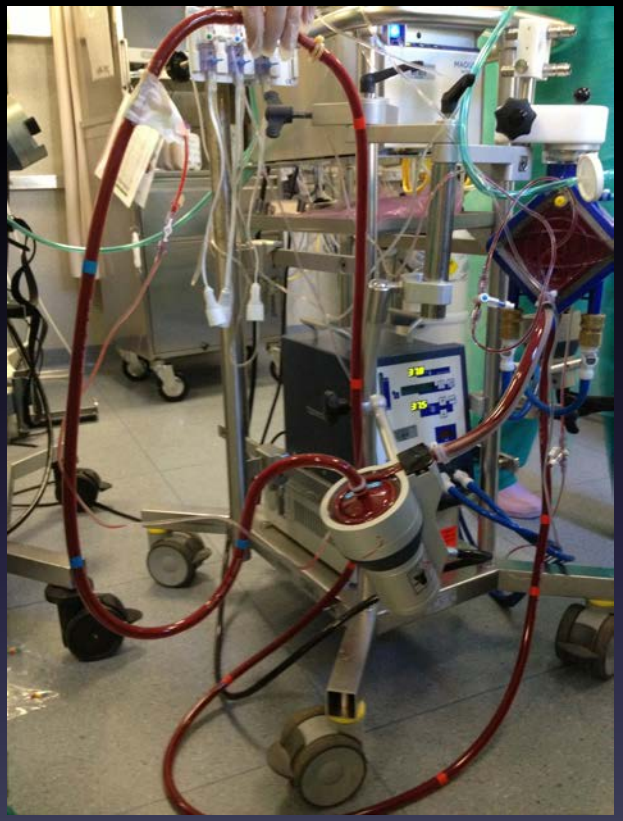
A Contributory Factor in the Development of Ventilator-Associated Pneumonia?

Primrose P. Freestone, PhD; Robert A. Hirst, PhD; Sara M. Sandrini, PhD; Fathima Sharaff, MSc; Helen Fry, MD; Stefan Hyman; and Chris O'Callaghan, DM, PhD



CHEST 2012; 142(5):1200–1210

Poor ability to use equipment and techniques developed for human applications



Duration of the Experiment

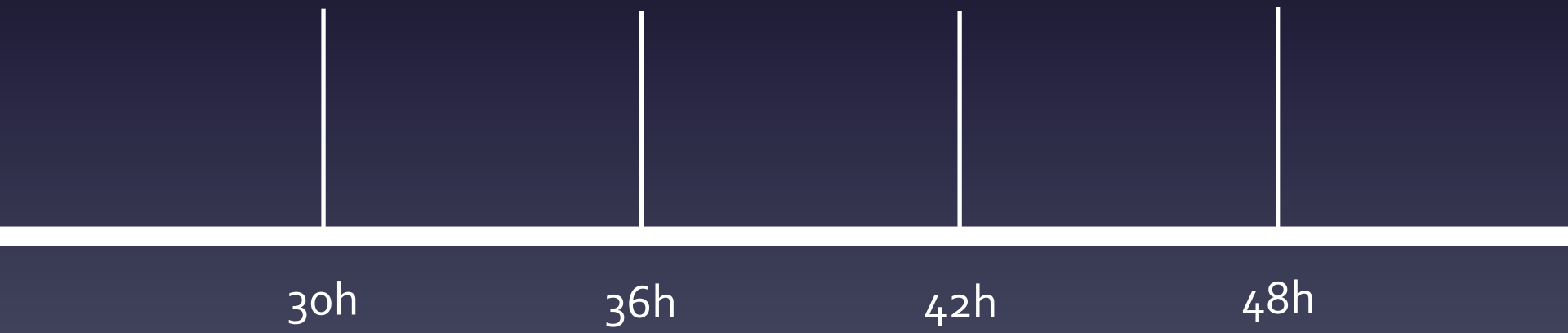
Start
Invasive MV

6h

12h

18h

24h


30h
36h
42h
48h

54h

60h

66h

72h

ASK THE EXPERTS

Are we beginning to believe that we can go so low in the mammalian species and still obtain valid information?

Would you as a critical care physician accept data from a drug study on an intensive care patient who was not only not resuscitated with fluid but who did not even have blood pressures and heart rates monitored?

Traber, Daniel L. PhD, FCCM
University of Texas Medical Branch (UTMB)
Crit Care Med 27(2), 1999, pp 255-256

It is rather unreasonable to believe that results from small-animal studies – started right after breakfast and completed in time for dinner – could be safely translated into valuable therapies for critically ill patients

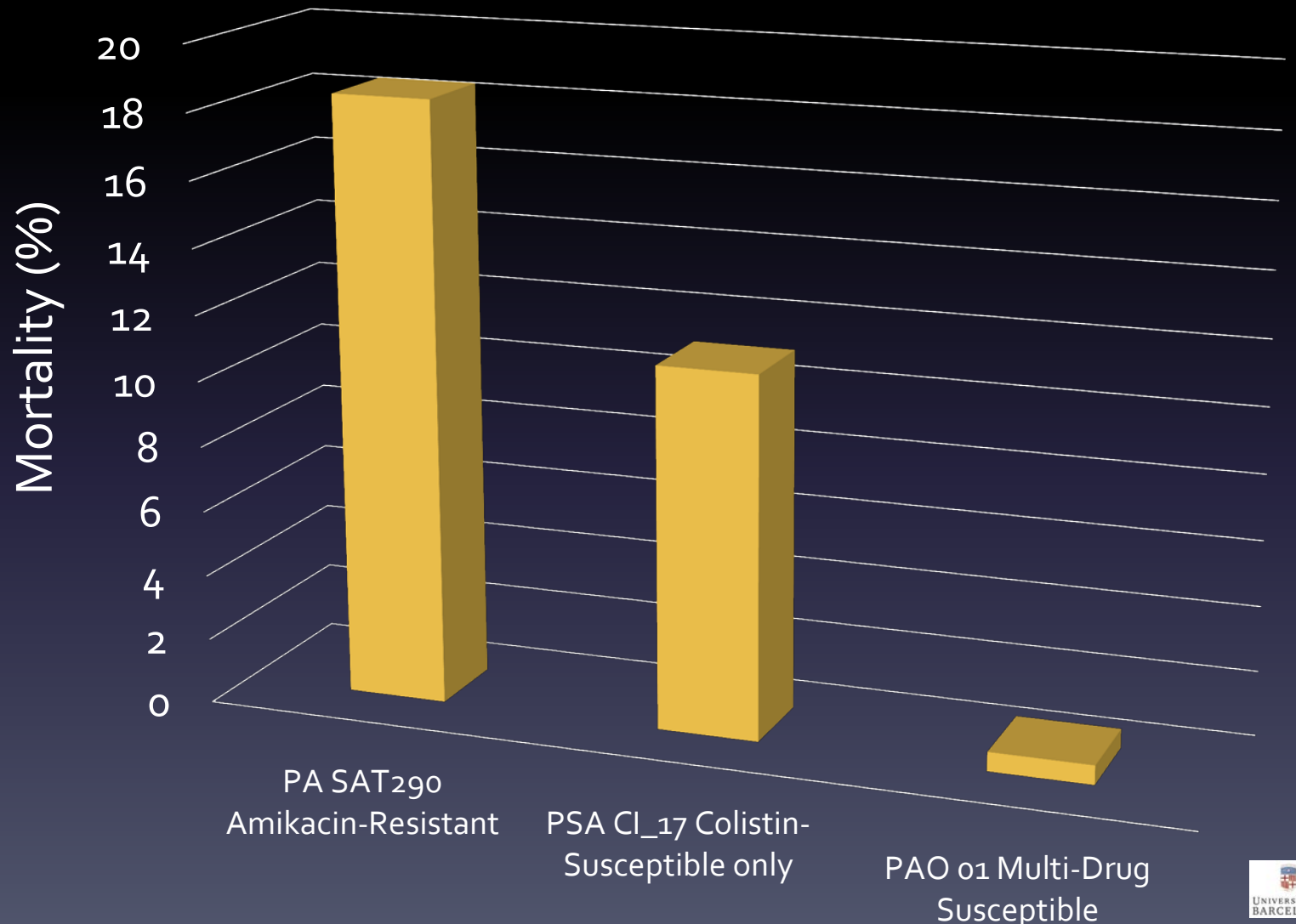
Theodor Kolobow, MD
Division of Pulmonary and Cardiac Assist Devices
National Institutes of Health, Bethesda, MD

VENTILATED ANIMAL MODELS OF P.AERUGINOSA PNEUMONIA

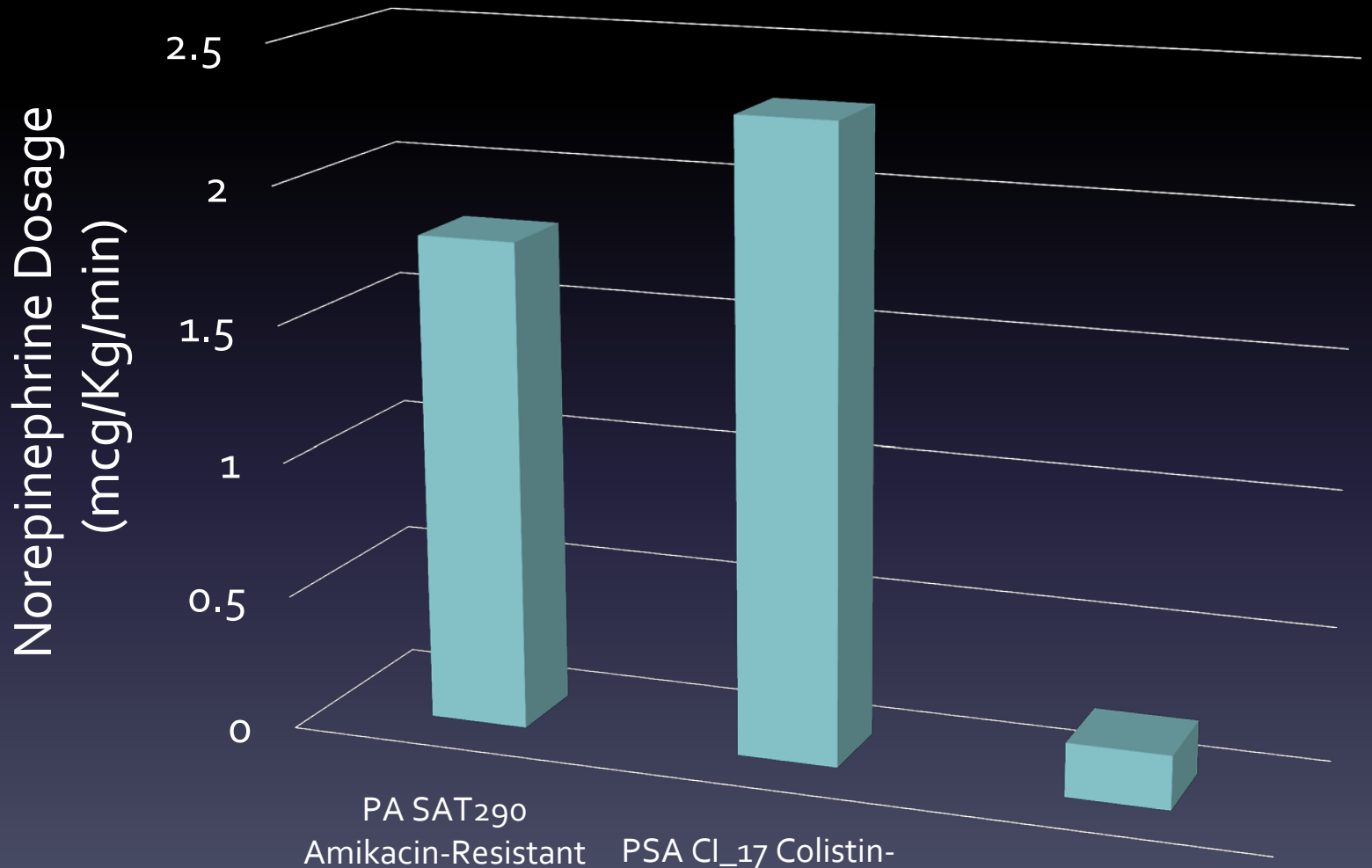
Features To Carefully Consider

- Burden of bacterial inoculum
- *Via* of Inoculation
- Animal position during inoculation
- Bacterial strain
- Pathogen antimicrobials susceptibility
- Type of animals
- Length of mechanical ventilation
- Severity of the disease

P.aeruginosa Strains and 24-h Mortality



P.aeruginosa Strains and 24-h Norepinephrine Requirements



1980s Johanson's Model

Johanson WG Jr, Holcomb JR, Coalson JJ.Experimental diffuse alveolar damage in baboons.
Am Rev Respir Dis. 1982 Jul;126(1):142-51.

Higuchi JH, Coalson JJ, Johanson WG Jr. Bacteriologic diagnosis of nosocomial pneumonia in primates. Usefulness of the protected specimen brush.
Am Rev Respir Dis. 1982 Jan;125(1):53-7.

Crouch TW, Higuchi JH, Coalson JJ, Johanson WG Jr.Pathogenesis and prevention of nosocomial pneumonia in a nonhuman primate model of acute respiratory failure.
Am Rev Respir Dis. 1984 Sep;130(3):502-4.

Johanson WG Jr, Seidenfeld JJ, Gomez P, de los Santos R, Coalson JJ. Bacteriologic diagnosis of nosocomial pneumonia following prolonged mechanical ventilation.
Am Rev Respir Dis. 1988 Feb;137(2):259-64.

Johanson's Model: Methods

- Adult baboons (10-24 Kg)
- Tracheally intubated, anaesthetized (diazepam, ketamine) and paralyzed (pancuronium)
- Oleic acid (0.04 ml/Kg) infused into the right atrium
- Animals on mechanical ventilation up to 10 days
- Lateral supine position and turned every 2h


Papio Papio


Papio Cynocephalus

Oropharyngeal and Pulmonary Colonization

- Oropharyngeal flora similar to humans (α-hemolityc strep, Neisseria sp, Staph aureus)
- Pneumonia was confirmed at necropsy and through histology studies (bronchiole and alveolar space filling with PMN)
- Etiology identified through postmortem lung aspirate or ante mortem blood culture in the absence of another identifiable source of bacteremia

TABLE 2

BACTERIAL CAUSES OF PRIMATE NOSOCOMIAL PNEUMONIA

Pathogen	Number of Animals
Pseudomonas aeruginosa	3
Morganella morganii	1
Pasteurella multocida	2
Staphylococcus aureus	1
Proteus mirabilis	2
Escherichia coli	1
P. aeruginosa and *S. aureus*	1
M. morganii and *E. coli*	1
Total	12

Johanson's Model: Advantages and Limitations

- Closely reproduces human pathogenesis and etiology; yet, *P.aeruginosa* is not the only causative pathogen
- Pulmonary challenge highly variable
- High costs, labor intensive and time consuming (72 hours prior to VAP onset)
- Scarce animal availability
- Strict legislations, guidelines, and policies related to the care and use of research primates

Luna's Model: Methods

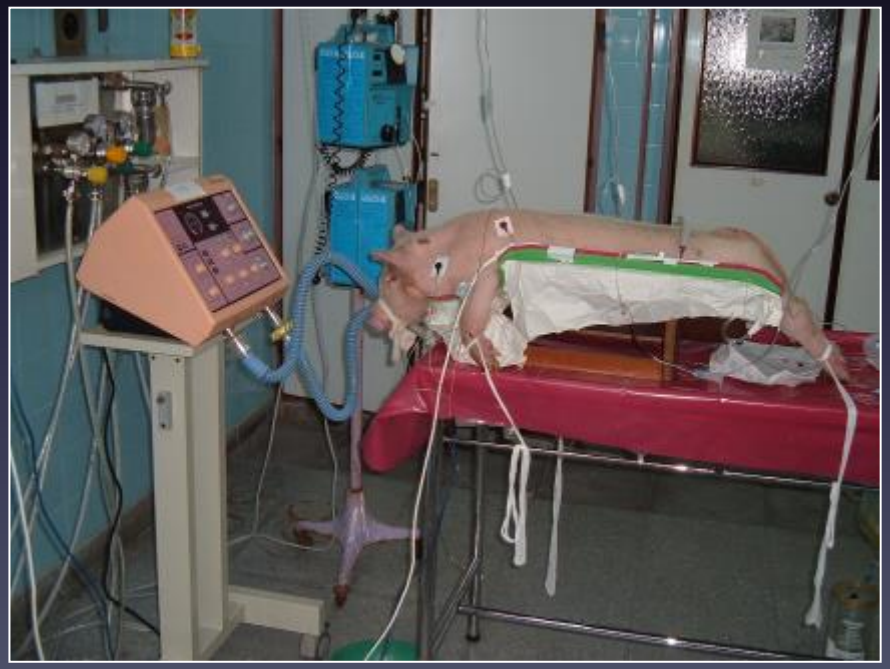
- Large White-Landrace female piglets (20±2 kg)
- MV up to 72 hours: VT 15 mL/Kg; PEEP 0 cmH2O
- Sedation/Analgesia: Midazolam/Fentanyl Paralysis: Pancuronium bromide
- Hemodynamic monitoring: Arterial catheter into the femoral artery; Pulmonary catheter through the internal jugular vein

Experimental Severe *Pseudomonas aeruginosa* Pneumonia and Antibiotic Therapy in Piglets Receiving Mechanical Ventilation*

Carlos M. Luna, Sebastián Baquero, Sebastián Gando, Juan Risso Patrón, Joaquín García Morato, Oriol Sibila, Rubén Absi, Angela Famiglietti, Carlos A. Vay, Florencia Von Stecher, Carlos Agustí and Antoni Torres

Chest 2007;132;523-531

- 15 ml of a 10^6 cfu/mL suspension of P. aeruginosa ATCC 27853 multi-susceptible inoculated into every lobe through a bronchoscope



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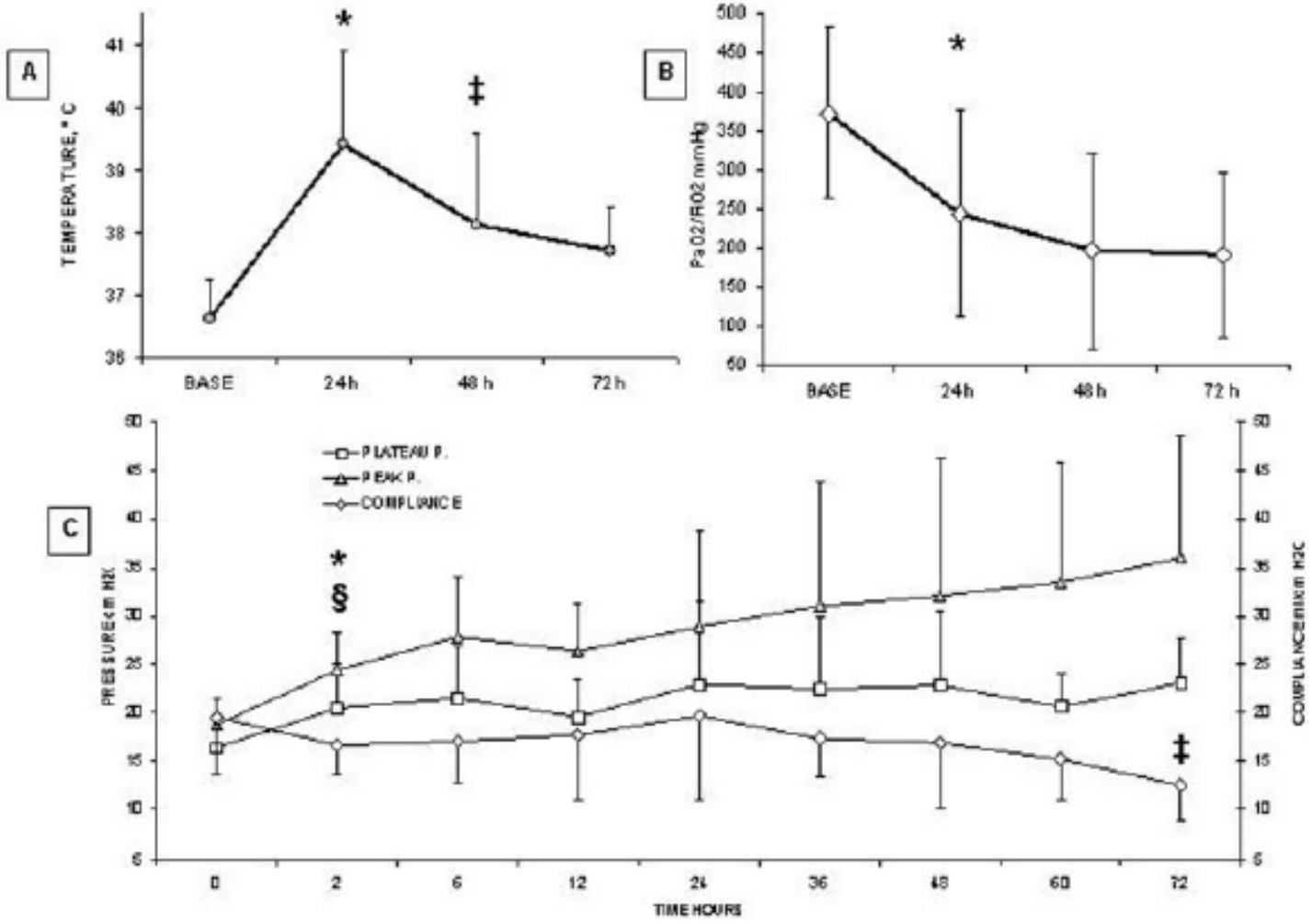
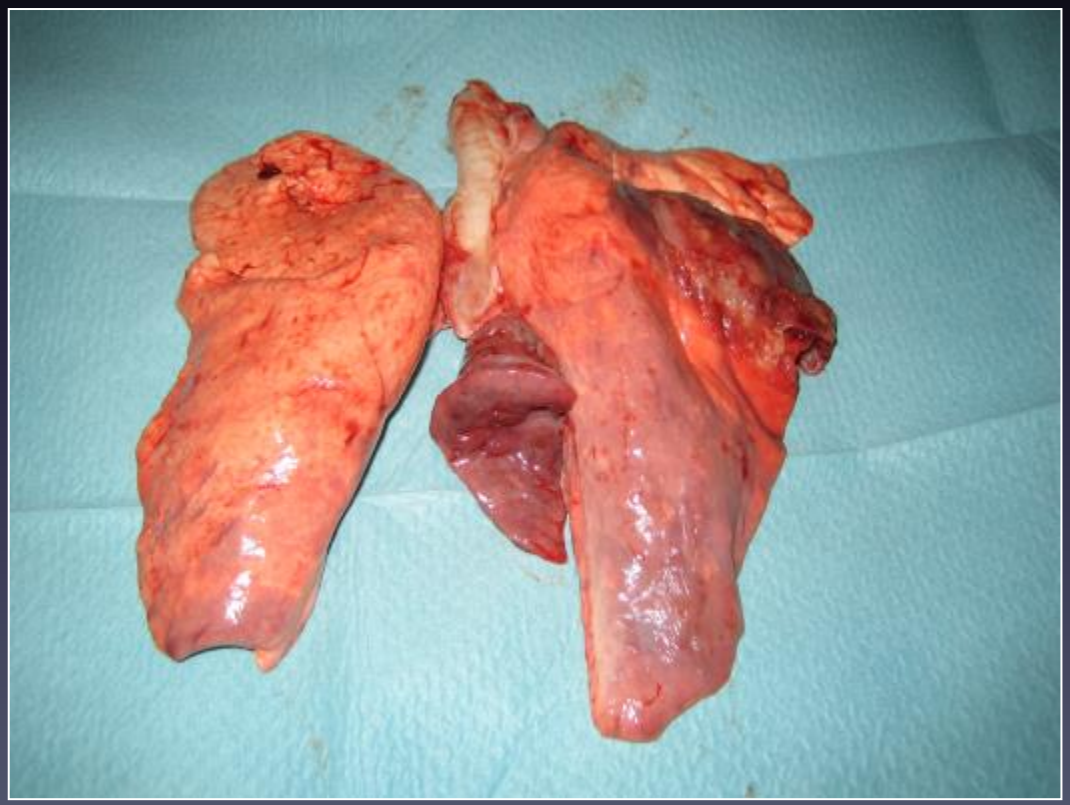
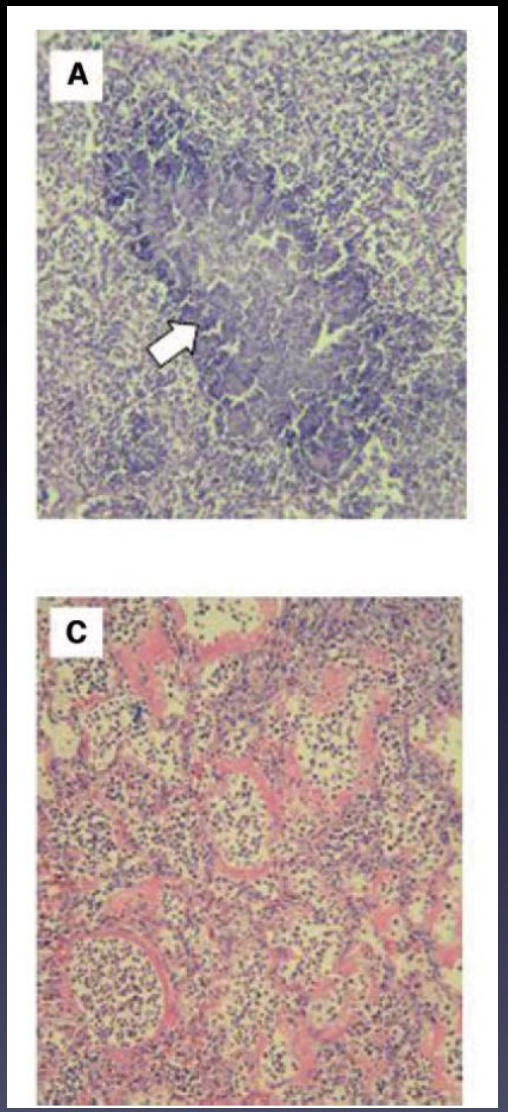


Microbiological/Histological Confirmation

- Upon autopsy, several specimens for bacteriologic and pathologic studies.





Luna's Model: Advantages and Limitations

- Rapid development of severe pneumonia, within 24 h
- Ideal model to study efficacy and safety of new pharmacological and non-pharmacological therapies
- Extensive pulmonary bacterial challenge

New Animal Model of *P.aeruginosa* VAP

A Novel Porcine Model of Ventilator-associated Pneumonia Caused by Oropharyngeal Challenge with *Pseudomonas aeruginosa*

Gianluigi Li Bassi, M.D., Ph.D., Montserrat Rigol, D.V.M., Ph.D., Joan-Daniel Marti, R.P.T., Ph.D., Lina Saucedo, M.D., Otavio T. Ranzani, M.D., Ignasi Roca, Ph.D., Maria Cabanas, M.D., Laura Muñoz, B.Sc., Valeria Giunta, M.D., Nestor Luque, M.D., Mariano Rinaudo, M.D., Mariano Esperatti, M.D., Laia Fernandez-Barat, Ph.D., Miquel Ferrer, M.D., Ph.D., Jordi Vila, Ph.D., Jose Ramirez, M.D., Ph.D., Antoni Torres, M.D., Ph.D.

ANESTHESIOLOGY 2014; 120:1205-15

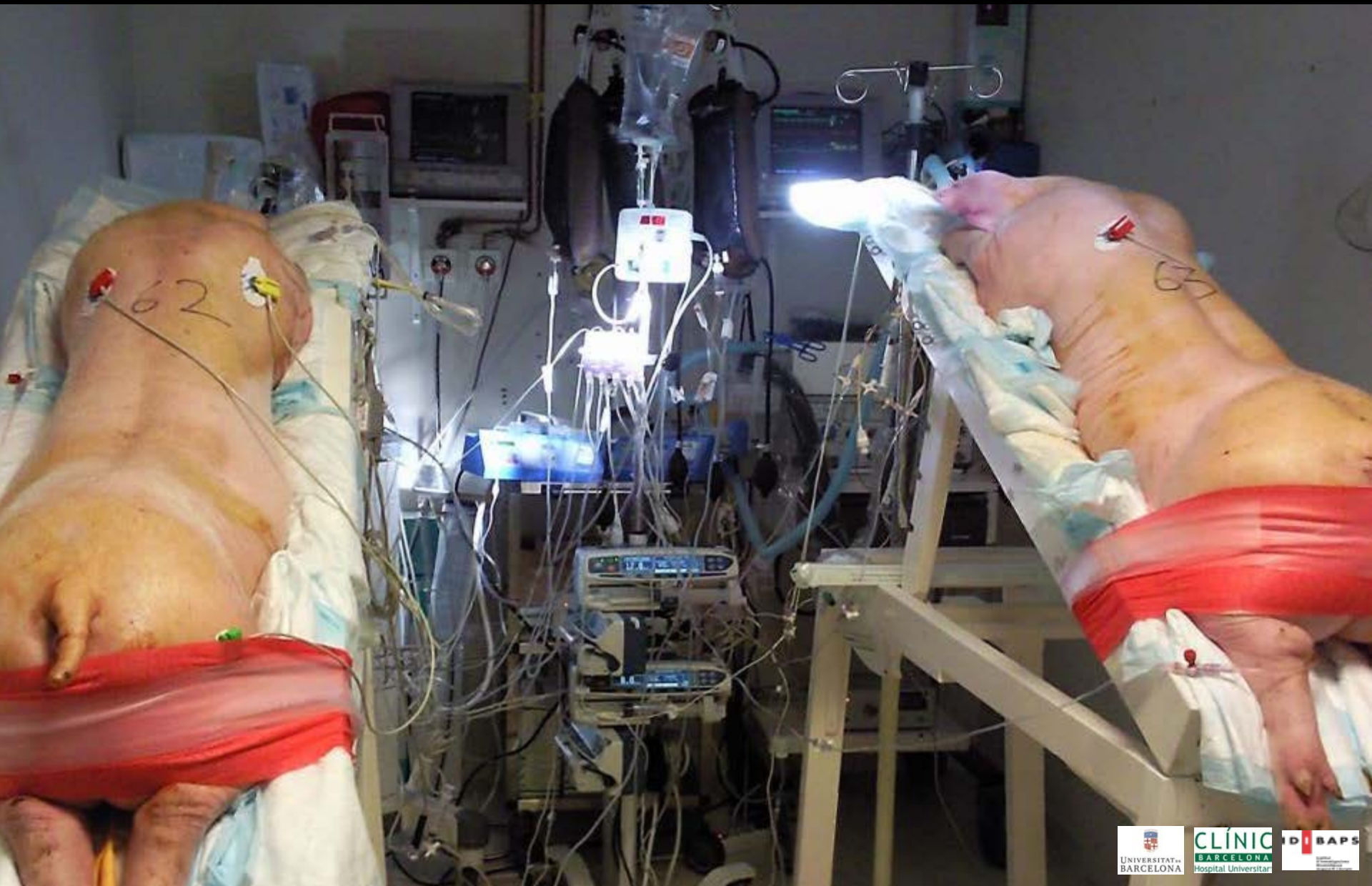
Our Goals

- To develop a novel model of *Pseudomonas aeruginosa* VAP to accurately reflect the key pathogenic mechanisms of the disease and most prevalent human etiology.

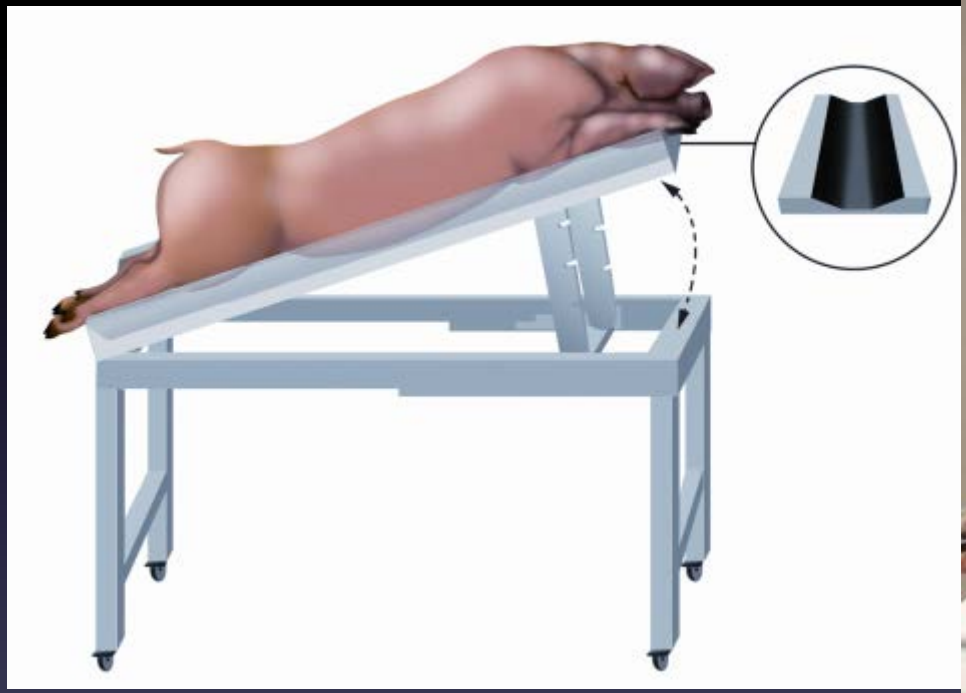
Methods

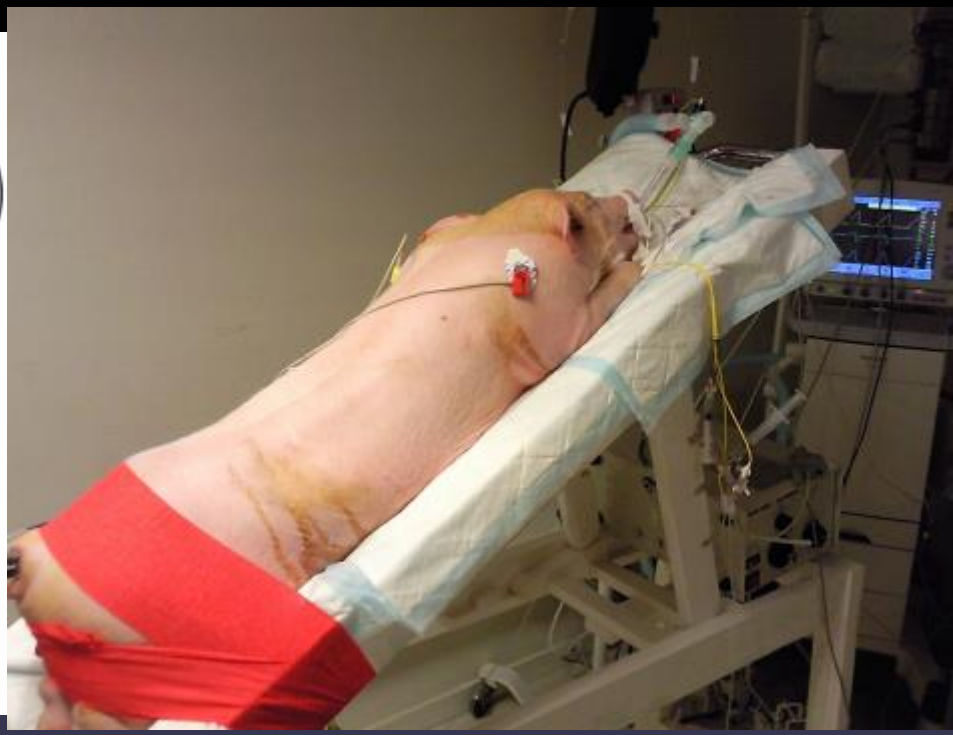
- Seven female Large White-Landrace pigs (Kg 30.5±1.8)
- Oro-Tracheal intubation (ETT n. 7.5 comprising HVLP cuff)
- MV up to 3 days: VT 10 mL/Kg; PEEP 0 cmH_2O
- Standard sedation, analgesia and paralysis
- Standard hemodynamic monitoring

Methods



Animal Position

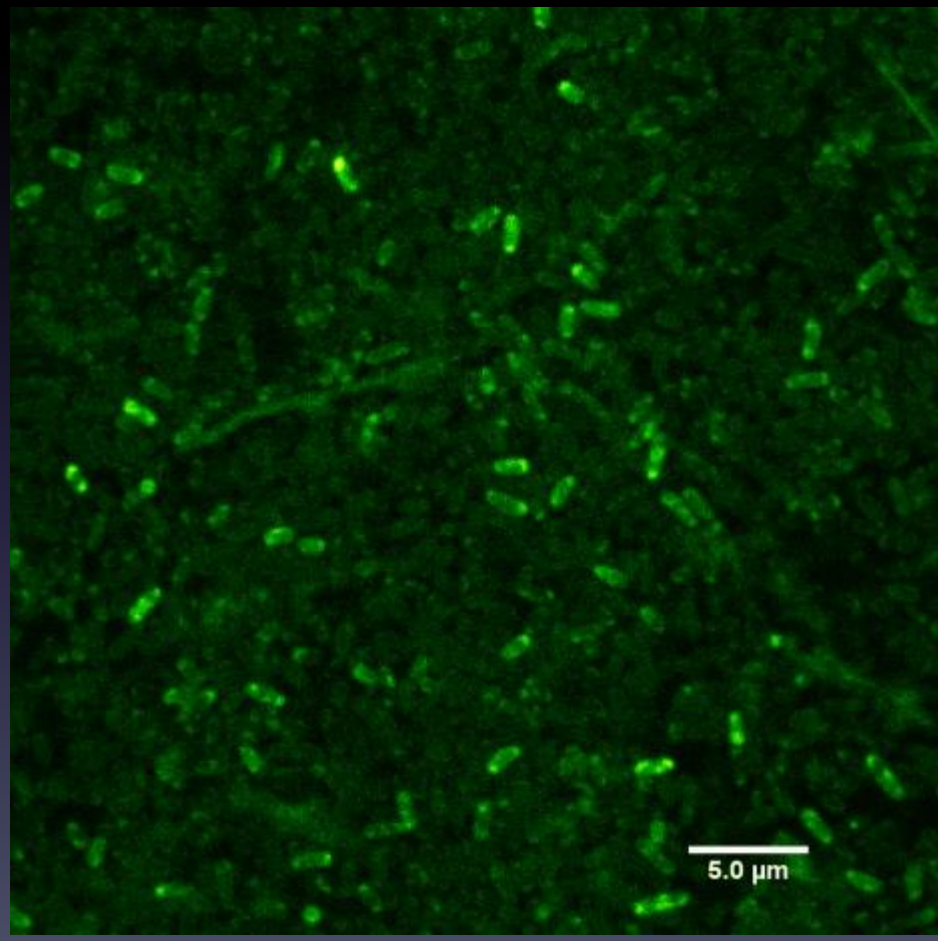




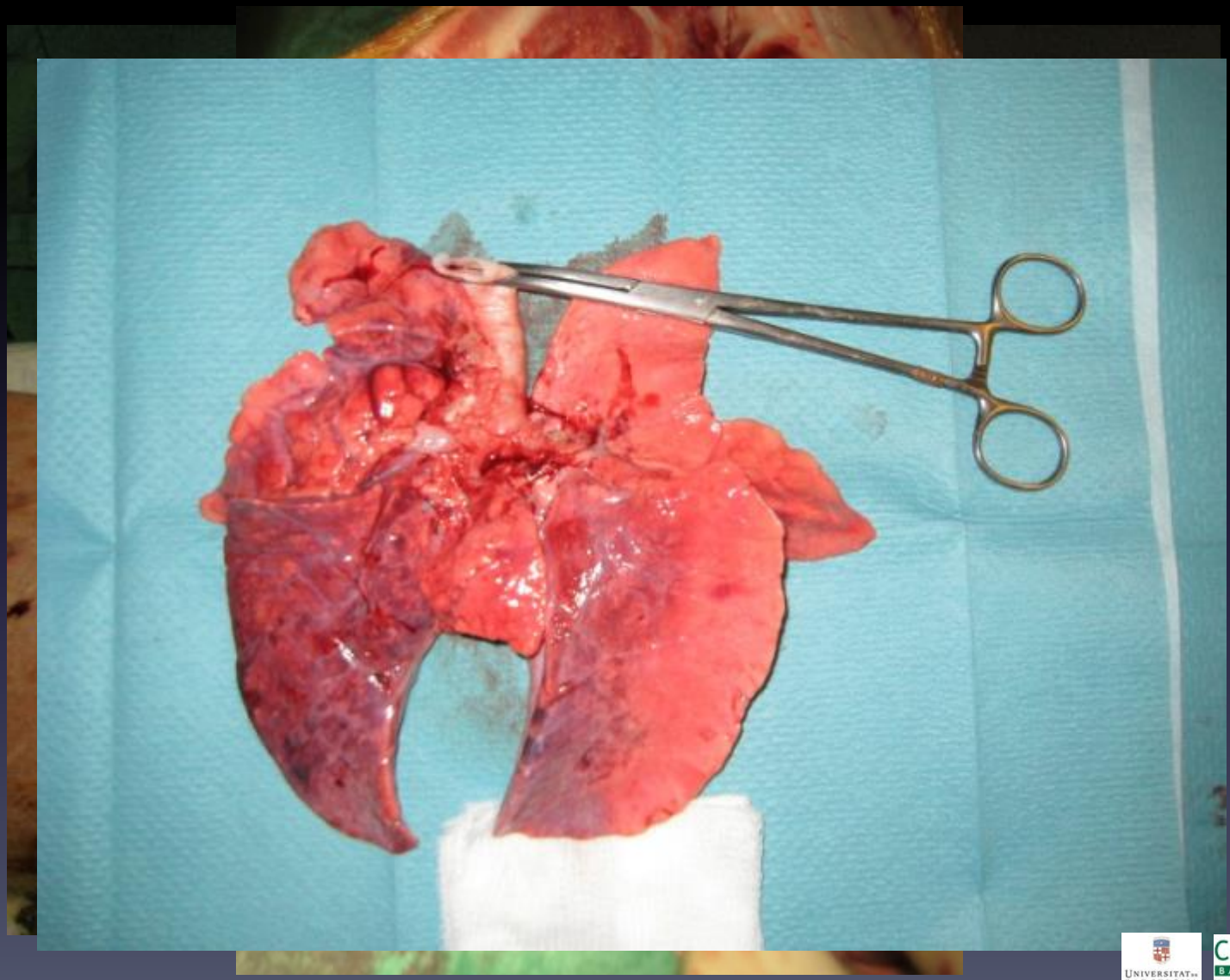
- Pigs were placed in the prone position, and the custom-made surgical bed, covered atop by an anti-slip fine ribbed rubber sheet, was oriented approximately 30 degrees in the anti-Trendelenburg position

Bacterial Inoculum

- After 4 and 8 hours of MV:
  - 5 mL of 10^7 cfu/mL culture of *PA 01* ATCC 27853 Ceftriaxone-resistant into the oropharynx
- Ceftriaxone to prevent colonization by endogenous pathogens

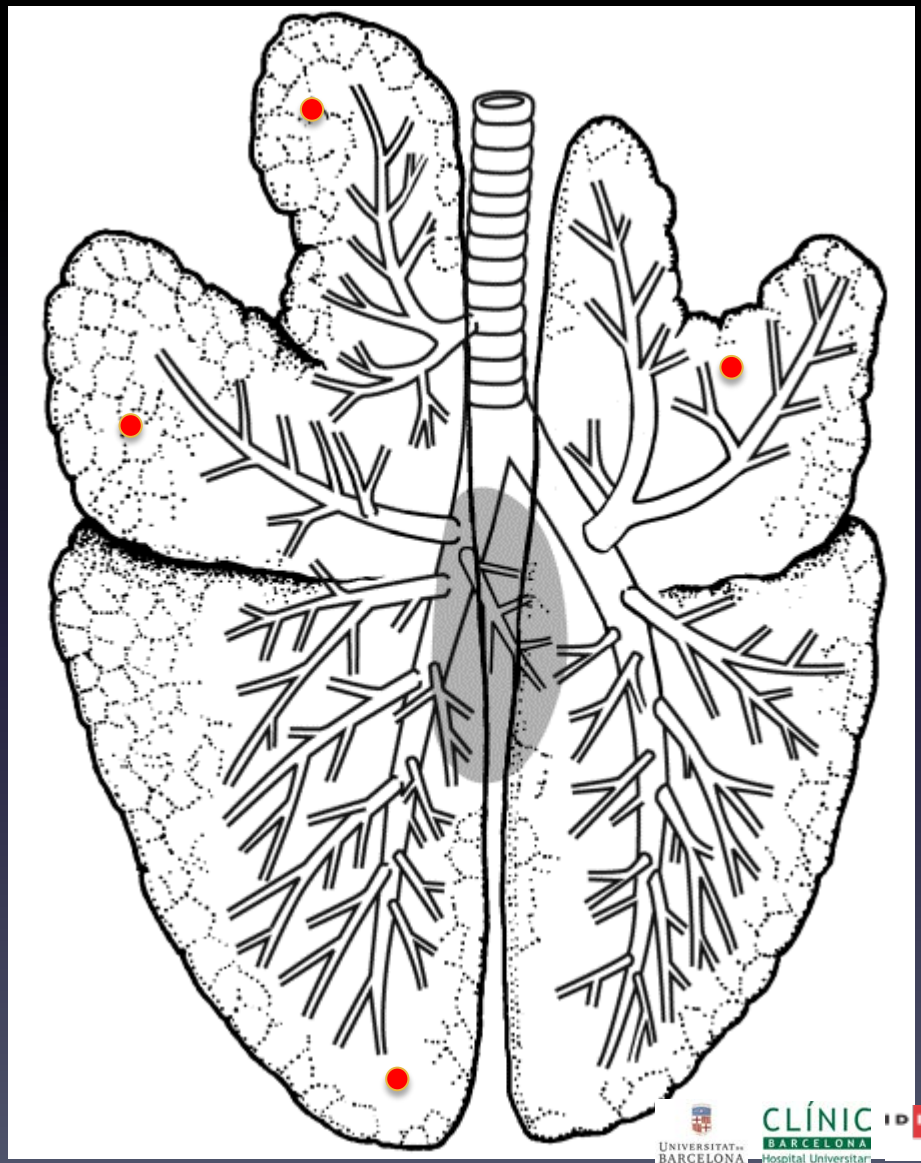


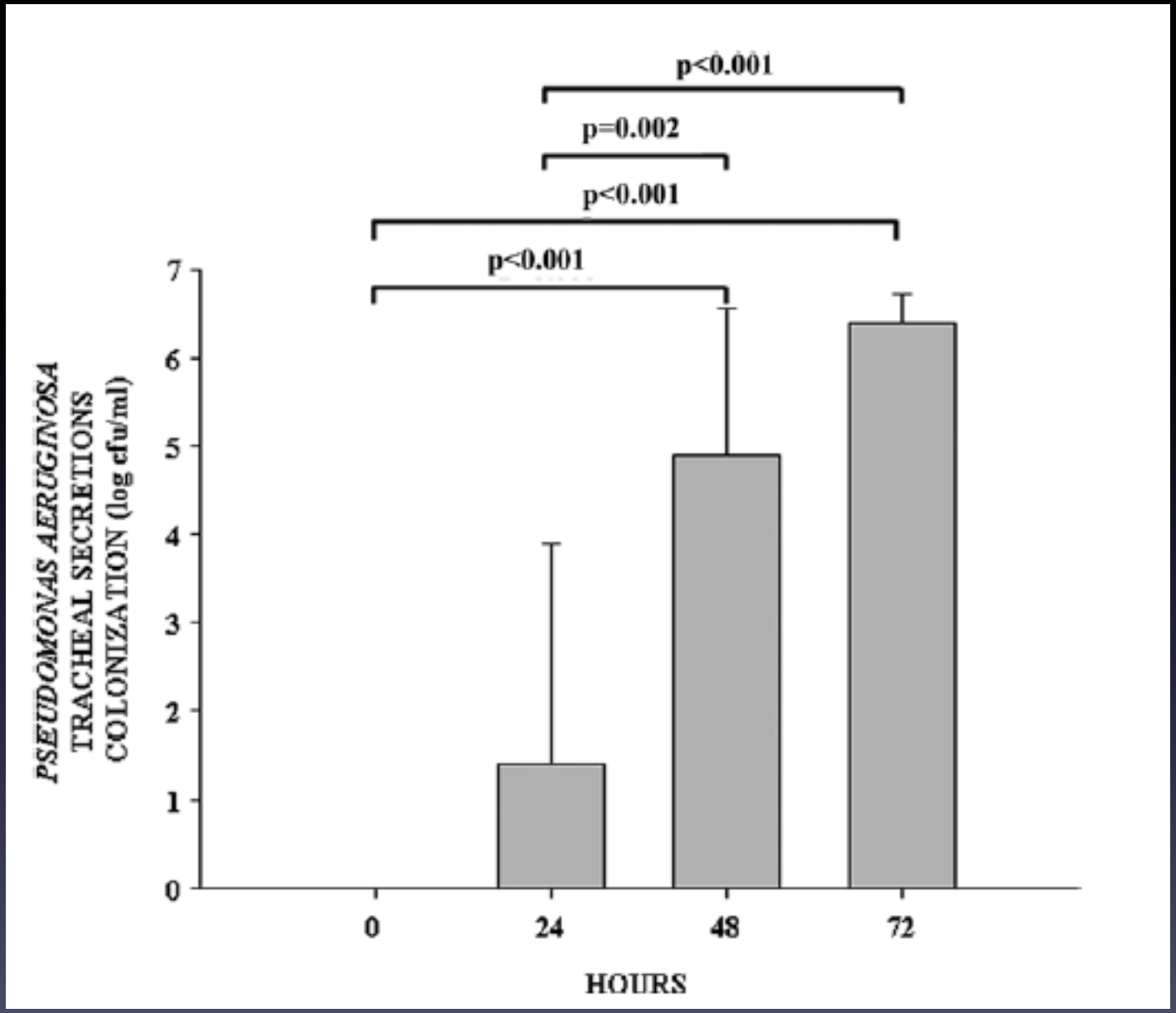
Autopsy



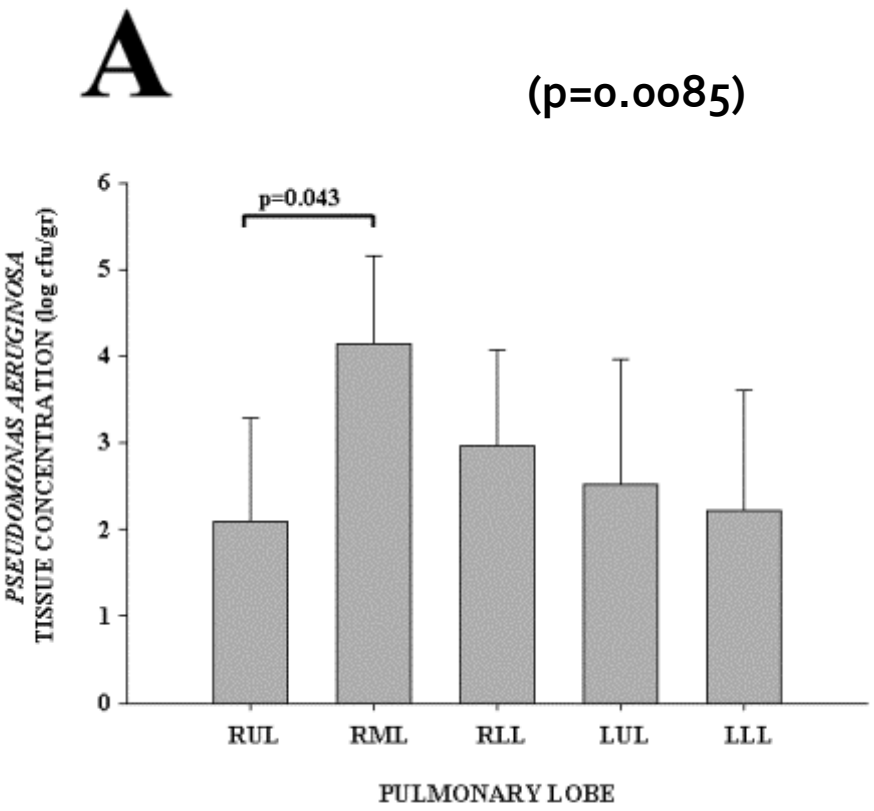
VAP Diagnosis

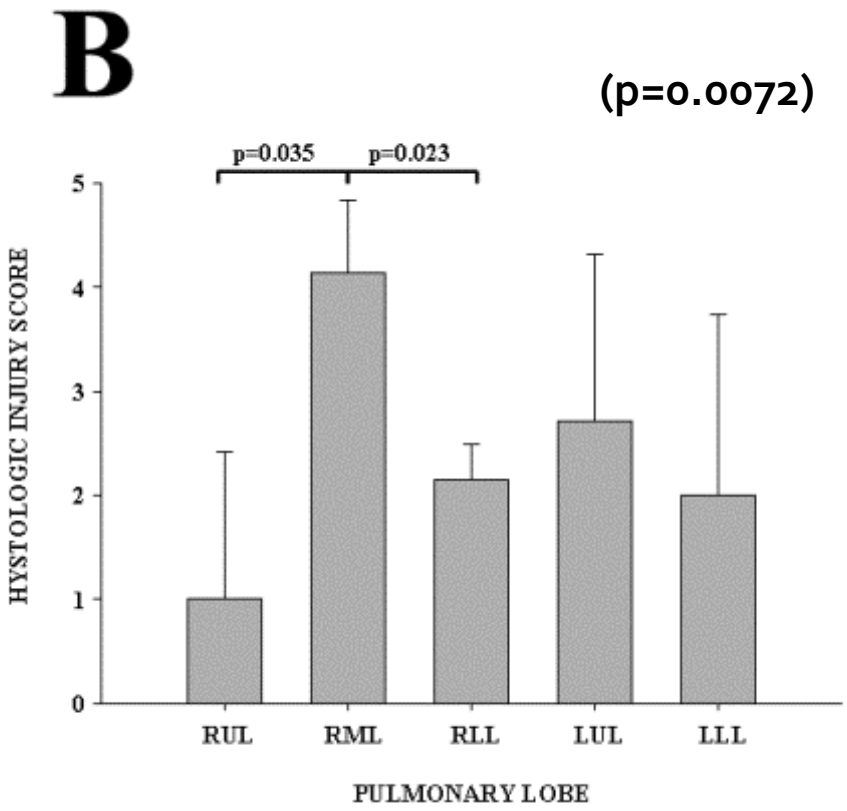
- Sampling was always performed in areas showing gross abnormalities, when present
- VAP was confirmed according to a histological injury score ≥ 3, associated with a quantitative *P. aeruginosa* lobar culture ≥ 3 log cfu/gr




p<0.001
p=0.002
p<0.001
p<0.001
PSEUDOMONAS AERUGINOSA
TRACHEAL SECRETIONS
COLONIZATION (log cfu/ml)
7
6
5
4
3
2
1
0
0
24
48
72
HOURS

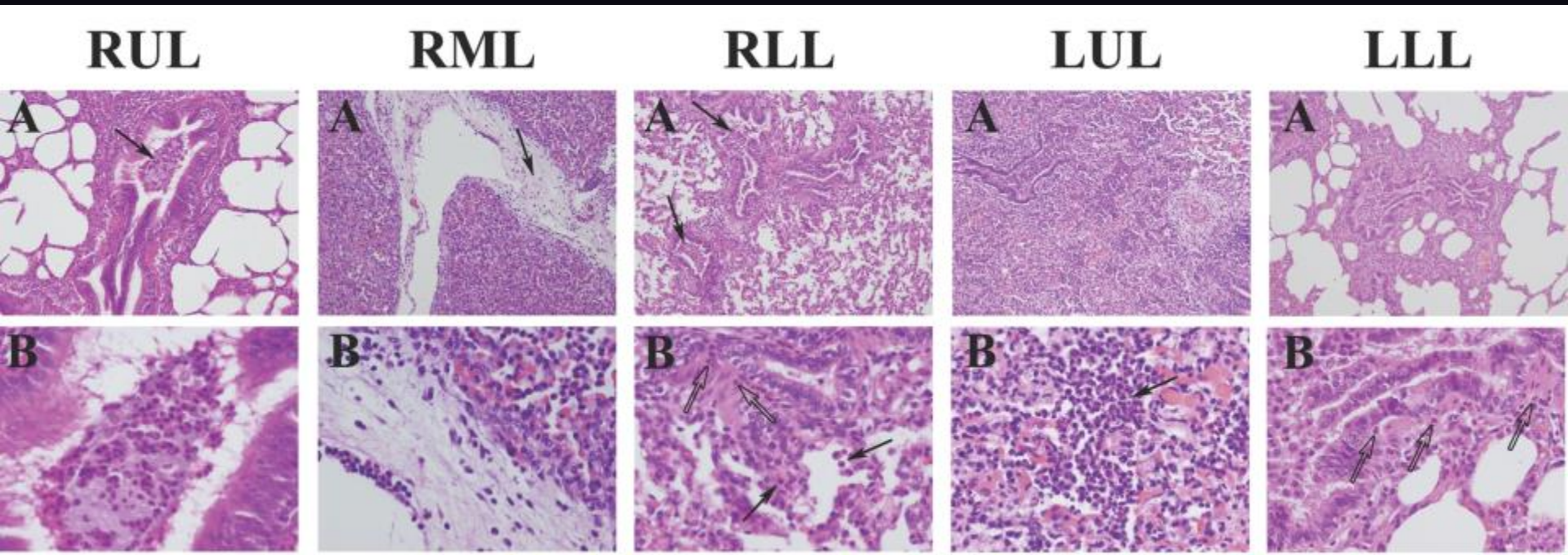
VAP was confirmed in 6 out of 7 pigs. In 55.56% of the cases, VAP developed in RML; in the RLL in 22.22%; in the LUL or LLL in 11.11%; and never in the RUL (p=0.032).





Characteristic histological pattern of median infection-related lung injury score

1	4	2	3	2
Purulent Mucus Plugging	Confluent Pneumonia	Bronchiolitis	Pneumonia	Bronchiolitis

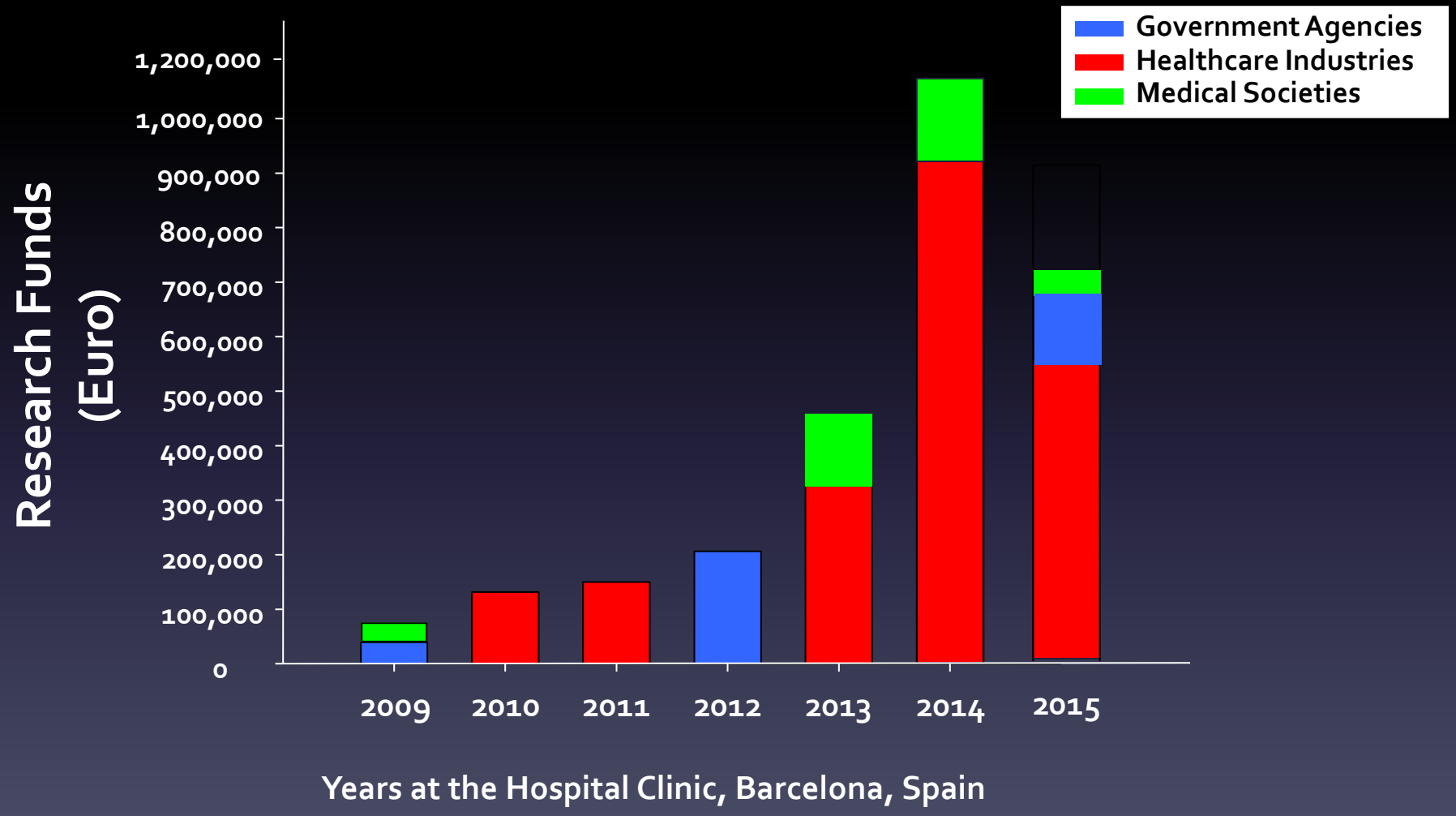


Li Bassi's Model: Advantages and Limitations

- Pulmonary aspiration of oropharyngeal secretions colonized by *P. aeruginosa*.
- Gravity-dependent dissemination of the infection
- VAP was associated neither with severe systemic clinical signs of infection nor with hemodynamic collapse.
- High Costs, labor intensive and time consuming (48-72 hours prior to VAP onset)
- Ideal to study pathophysiologic mechanisms of the disease, diagnostic strategies and to test effectiveness and safety of preventive strategies

APPLICATIONS

Grants/Funding



Grants/Funding Sources

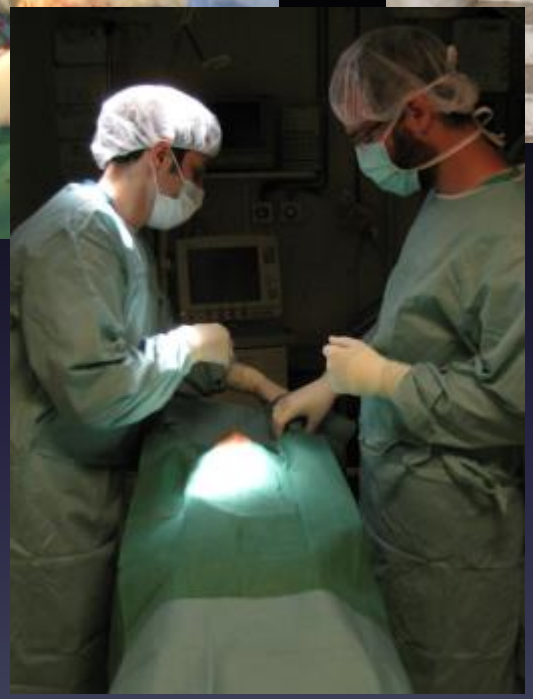
Healthcare Industry	Protocol
Bayer, Leverkusen, Germany 	THE EFFECTS OF AEROSOLIZED AMIKACIN IN PIGS WITH SEVERE PSEUDOMONAS AERUGINOSA PNEUMONIA
Toray Industries, Tokyo, Japan 	EFFECTS OF HEMOPERFUSION WITH POLIMIXIN B-IMMOBILIZED FIBER COLUMN ON ENDOTOXIN CLEARANCE IN A MODEL OF SEVERE PSEUDOMONAS AERUGINOSA PNEUMONIA
Cardeas Pharma, Seattle, WA, USA 	NEBULIZED FOSFOMYCIN AND AMIKACIN IN AN EXPERIMENTAL MODEL OF SEVERE PNEUMONIA INDUCED BY P. AERUGINOSA IN VENTILATED PIGS
Medimmune, Gaithersburg, MD, USA 	EFFICACY OF MEDI3902 IN THE PREVENTION AND TREATMENT OF PSEUDOMONAS AERUGINOSA VENTILATOR-ASSOCIATED PNEUMONIA
Cubist, Lexington, MA, USA 	THE EFFECTIVENESS OF CEFTOLOZANE/TAZOBACTAM IN COMPARISON WITH PIPERACILLIN/TAZOBACTAM IN AN EXPERIMENTAL MODEL OF SEVERE PSEUDOMONAS AERUGINOSA PNEUMONIA

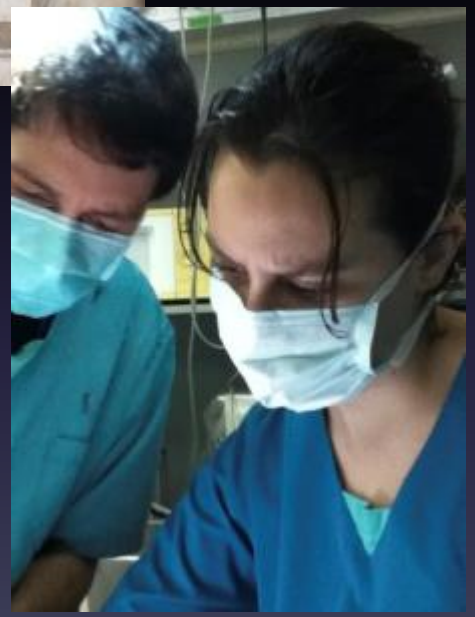
Conclusions

- Animal models of pneumonia are essential tools:
  - To elucidate pathogenesis of lung infections
  - To characterize pathogen virulence factor, innate and adaptative immune response
  - To test efficacy and safety of novel therapies
- Several animal models of *P.aeruginosa* pneumonia represent milestones in this field and constitute the basis for future methodological improvements

Conclusions 2

- The pig model is the most appropriate model of *P.aeruginosa* pneumonia treated in the ICU (particularly VAP)
  - Close anatomical similarities with the human
  - Good survival, irrespective of the severity of the infection
  - Possibility of prolonged mechanical ventilation
  - Reproduction of the main pathogenic mechanisms of the human disease





Thank You

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