Panton–Valentine leukocidin (PVL) Role in the Induction, Maintenance and Local Extension of Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) Rabbit Osteomyelitis

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Background

- ◆ **The role of PVL in CA-MRSA pathogenicity remains controversial and might depend on the infection site and/or experimental model**

- ◆ **Osteomyelitis has long been recognized as a major clinical syndrome of invasive *S. aureus* disease.**

- ◆ **The impact of PVL on the course of acute osteomyelitis in children and young adults**

  - ❖ **was already suspected during the pre-antibiotic era**
    (Specific immunity in acute staphylococcal osteomyelitis. Valentine and Butler Lancet 1939;1: 973-8)

  - ❖ **was recently readdressed in the era of CA-MRSA**
    (CE Bocchini et al. Pediatrics 2006; 117:433-40 Dohin et al. Pediatr Infect Dis J. 2007; 26:1042-8)

Study Objective

◆ **Assess the specific role of PVL**

   in a CA-MRSA rabbit osteomyelitis model

◆ **By comparing the outcomes of infections caused by**

   ❖ **PVL-positive MRSA USA 300: LAC**

   ❖ **PVL-negative isogenic strain: LACΔ *pvl***

   **(kindly provided by Frank R. DeLeo)**
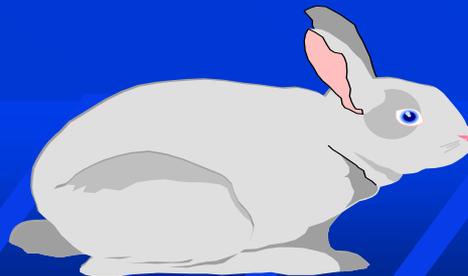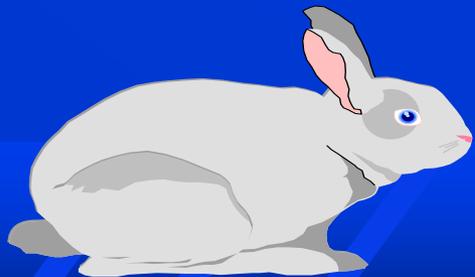
Rabbit osteomyelitis model
(Norden's model J Infect Dis 1970)

- **New Zealand white rabbits**

- **Intramedullary injection of a sclerosing agent (0.1 mL of 3% sodium tetradecyl sulfate) into the tibia, followed by 0.2 mL of inoculum**

- **Inocula: $8×10^5$ (low) or $4×10^8$ (high) CA-MRSA CFU (cultured in CCY and diluted in PBS)**

- **On D7 and D28, rabbits were sacrificed**
  - macroscopic findings: noted and photographed,
  - infected tibias were removed and bacteria in crushed bones were counted.
  - Serum samples : Anti PVLantibody and CRP titers
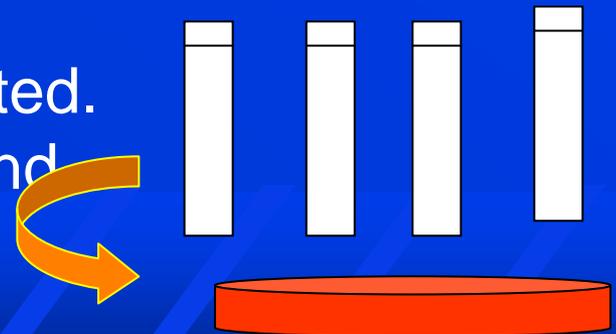
Imaging and histopathological examinations

- **Low inoculum:** Serial MRI (Philips INTERA 1.5T) performed on 6 rabbits (D7, D14, D21)

- **High inoculum:** Plain film +MRI + histopathological examination of 6 rabbits at the time of sacrifice (D7 or D28)
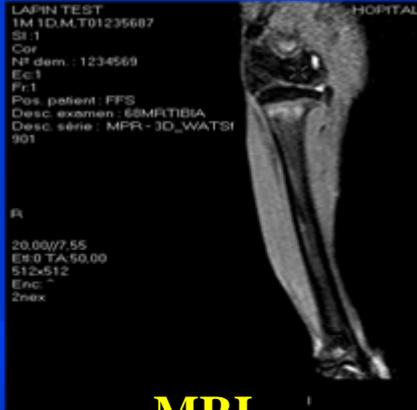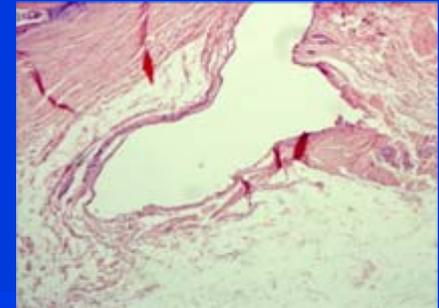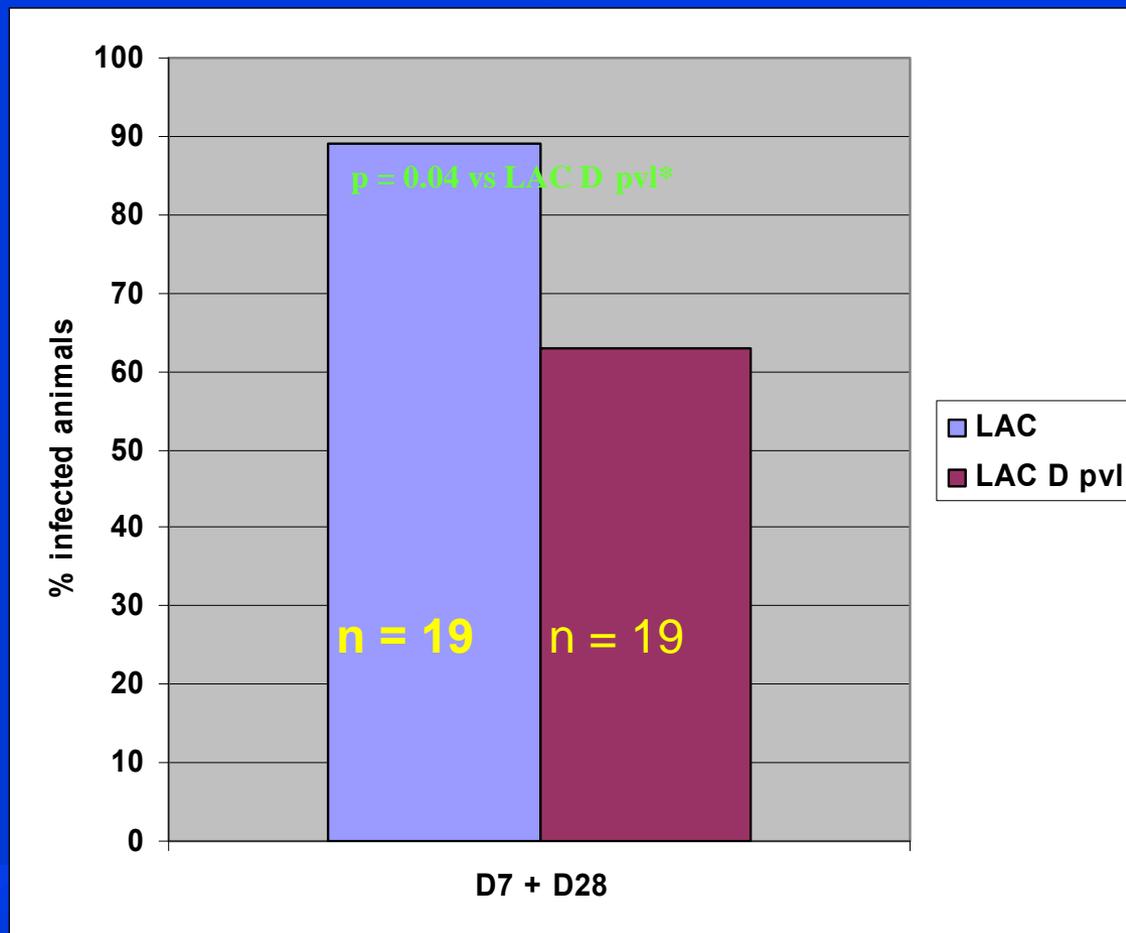

MRI

(C. Vallee)

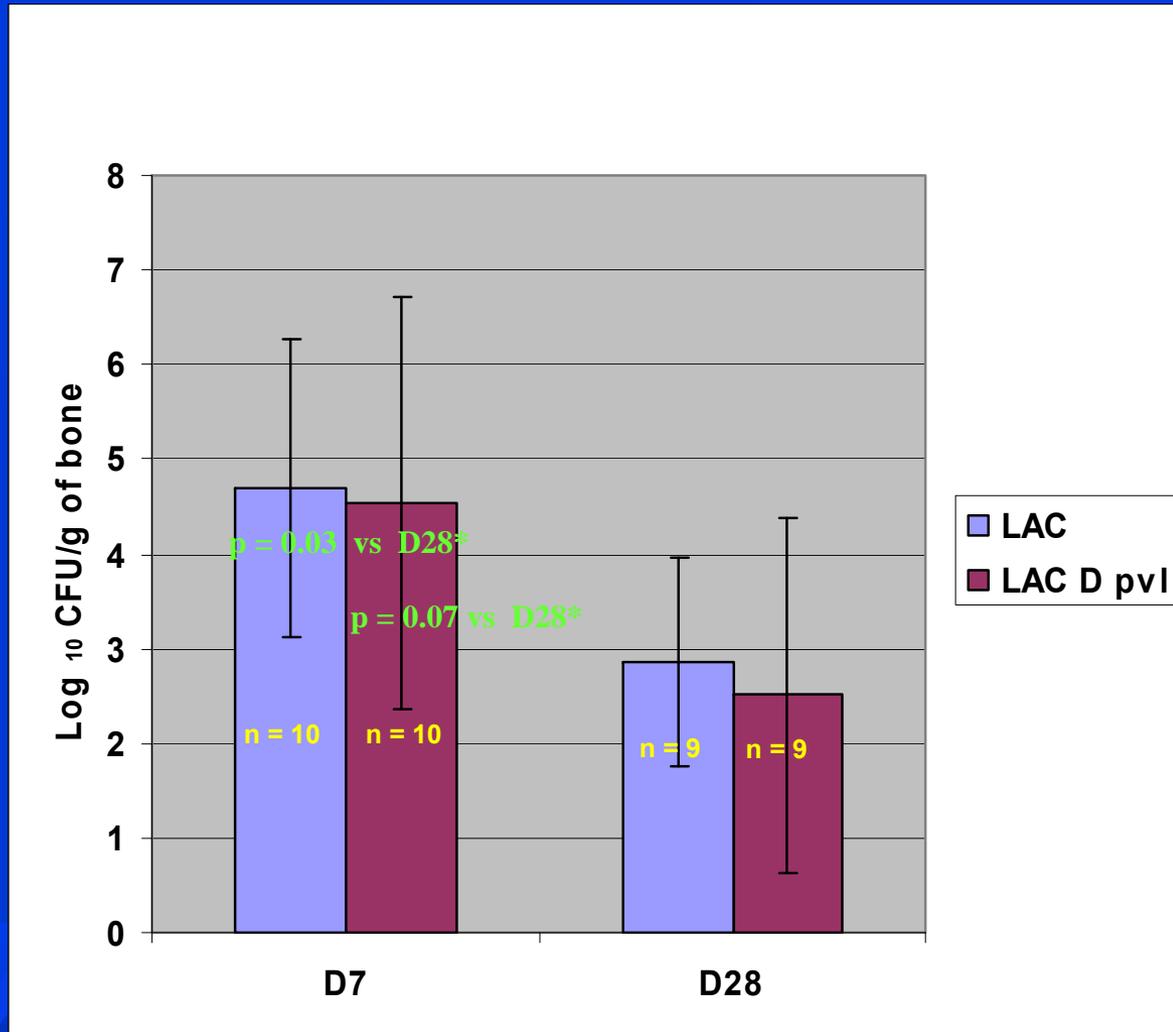Muscle
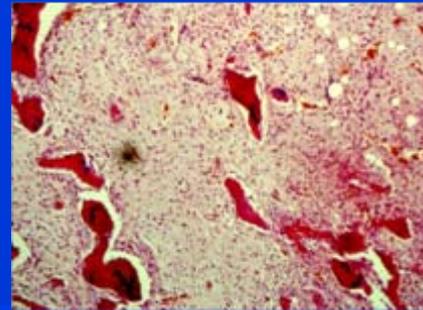
Bone marrow

(JF Cotte)

Joint space

Results
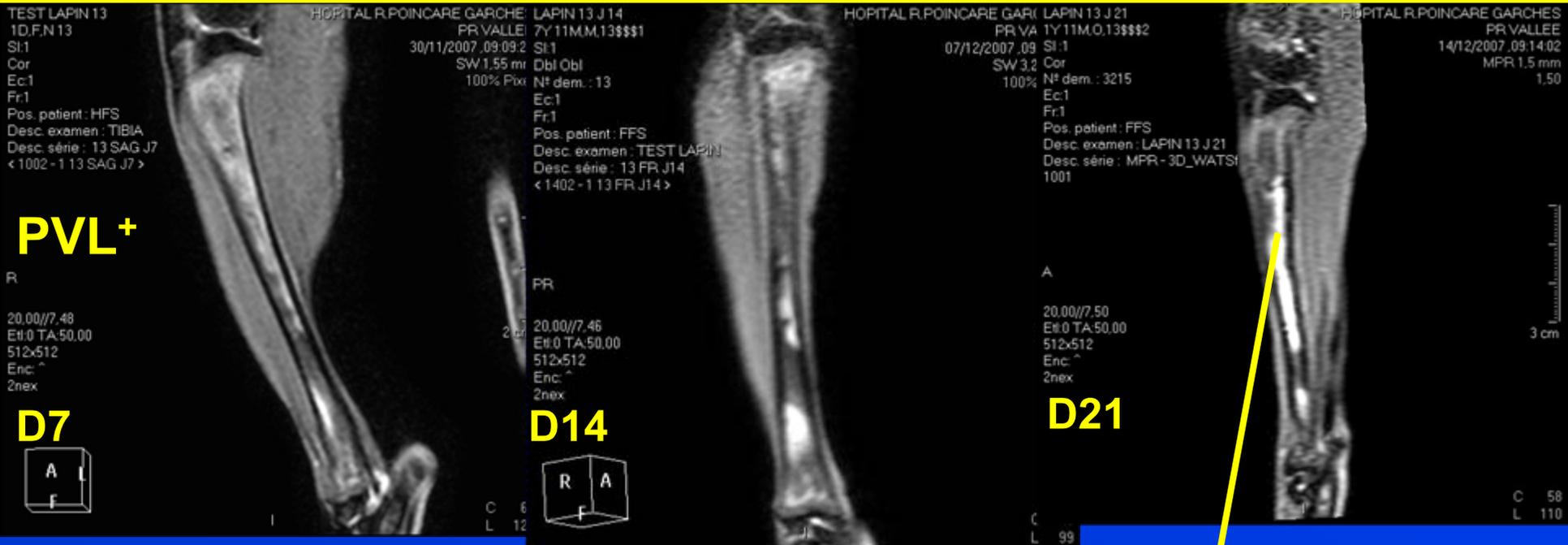## Low Inoculum (8 x 10^5 CA-MRSA CFU)

* Fisher's Exact test

Results
Low Inoculum (8×10^5 CA-MRSA CFU)

* Mann-Whitney non-parametric U-test

MRI PVL⁺

PVL⁺

D7

D14

D21

Control

$4.6 \log_{10} \text{CFU/g}$

D28

Lapin N° 13

MRI PVL⁻

D7

D14

D21

Control

6.0 log₁₀ CFU/g
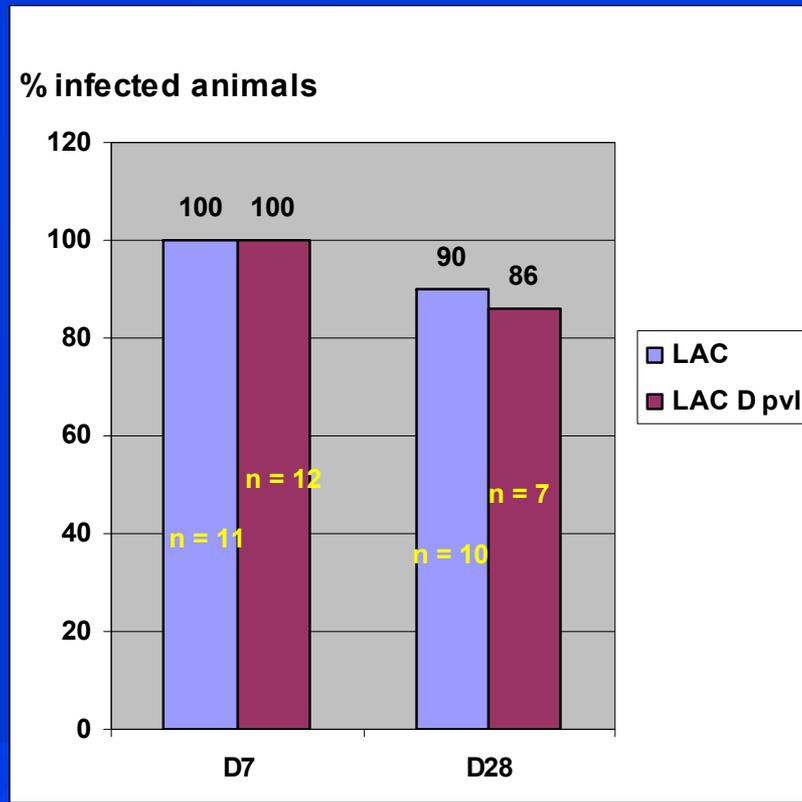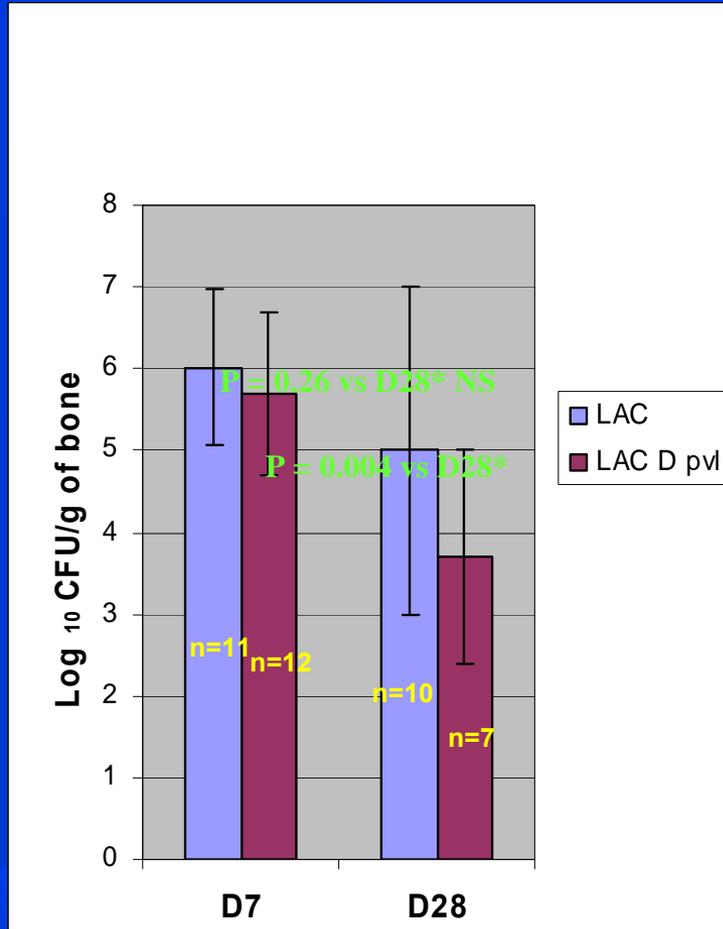
LapinN° 16

D28

Results
High Inoculum (4×10^8 CA-MRSA CFU)

Results
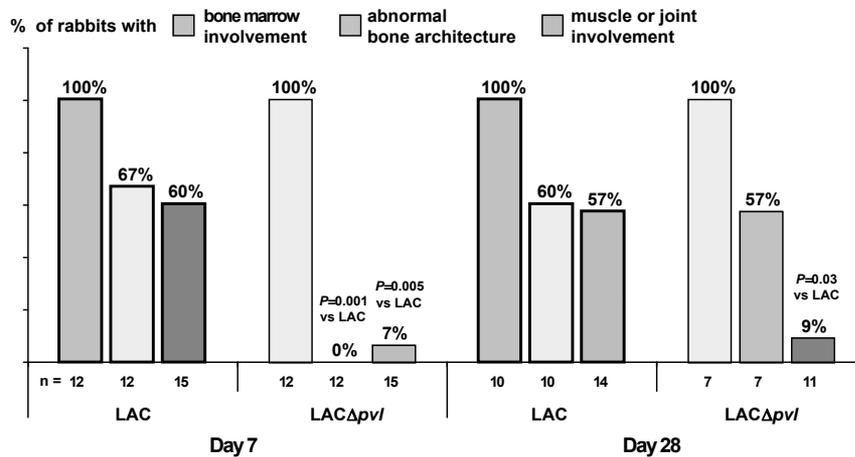## High Inoculum (4 x 10^8 CA-MRSA CFU)
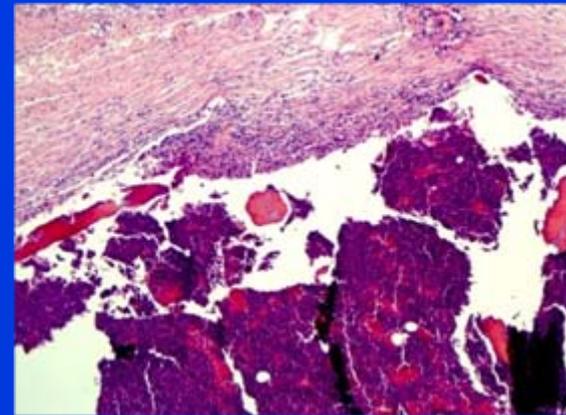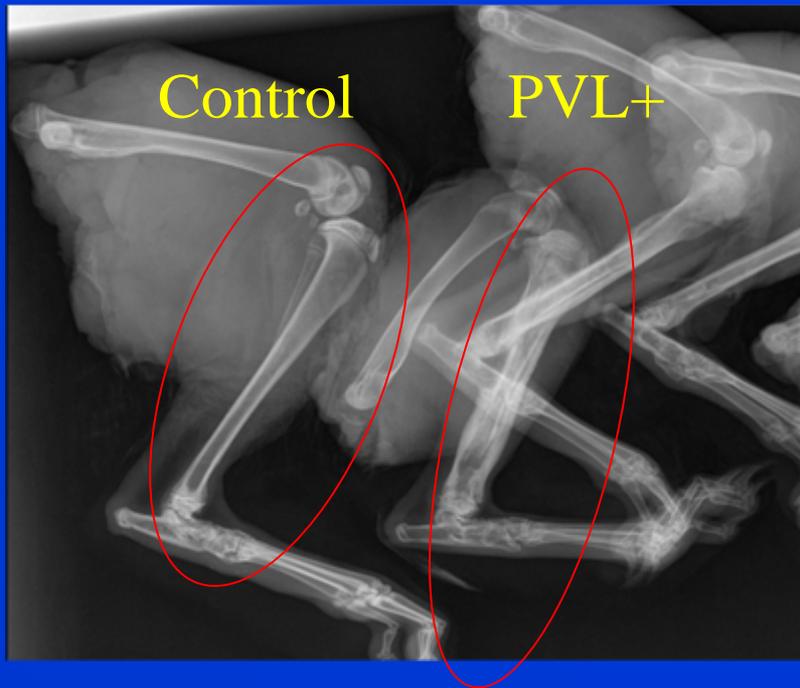

* Mann-Whitney non-parametric U-test

Results
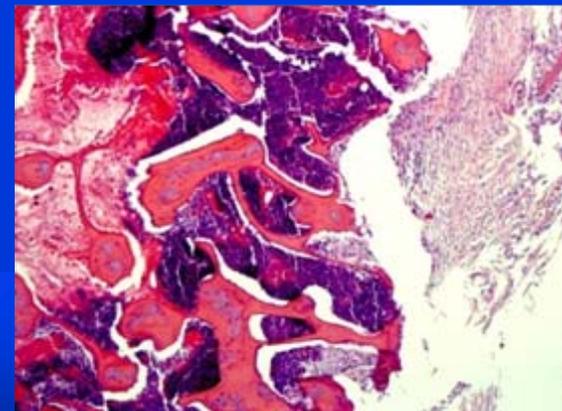## High Inoculum (4 x10^8 CA-MRSA CFU)
Macroscopic findings

High Inoculum (4×10^8 CA-MRSA)
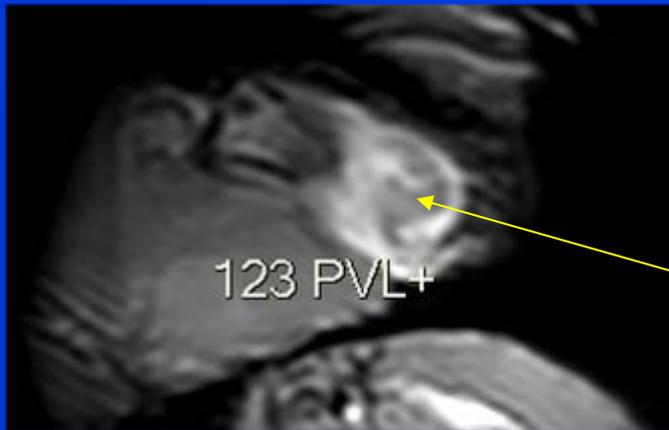## Radiographic and histological findings in a PVL[+]-infected rabbit on D28

Control   PVL+

Deformation and widening of the entire diaphysis
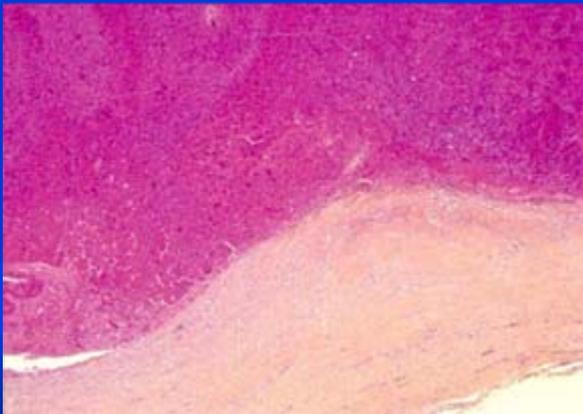
Bone

Abscess

Sequestrum

High Inoculum
MRI and histological findings in a PVL⁺ -infected rabbit on D28
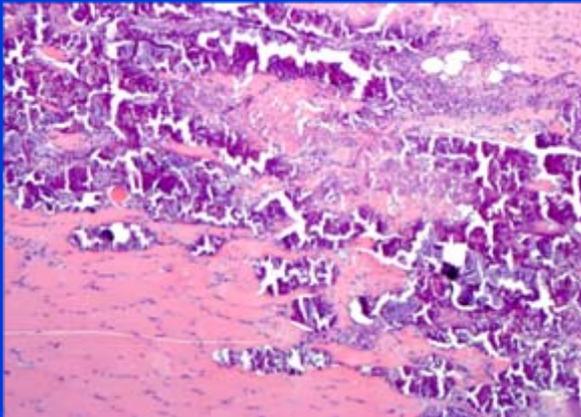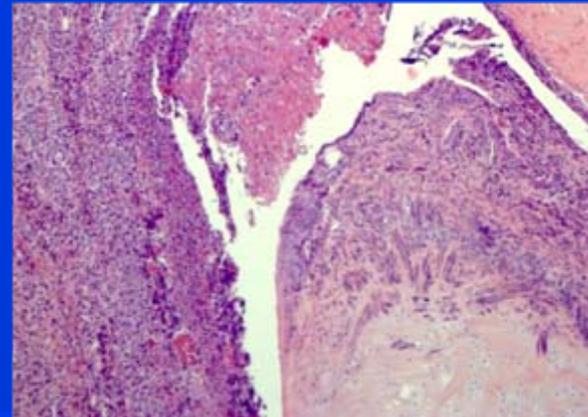

Soft tissue
Abscess
3x2x1(cm)

x10

x1

High Inoculum

MRI and histological findings in a PVL$^+$ - infected rabbit that died on D8
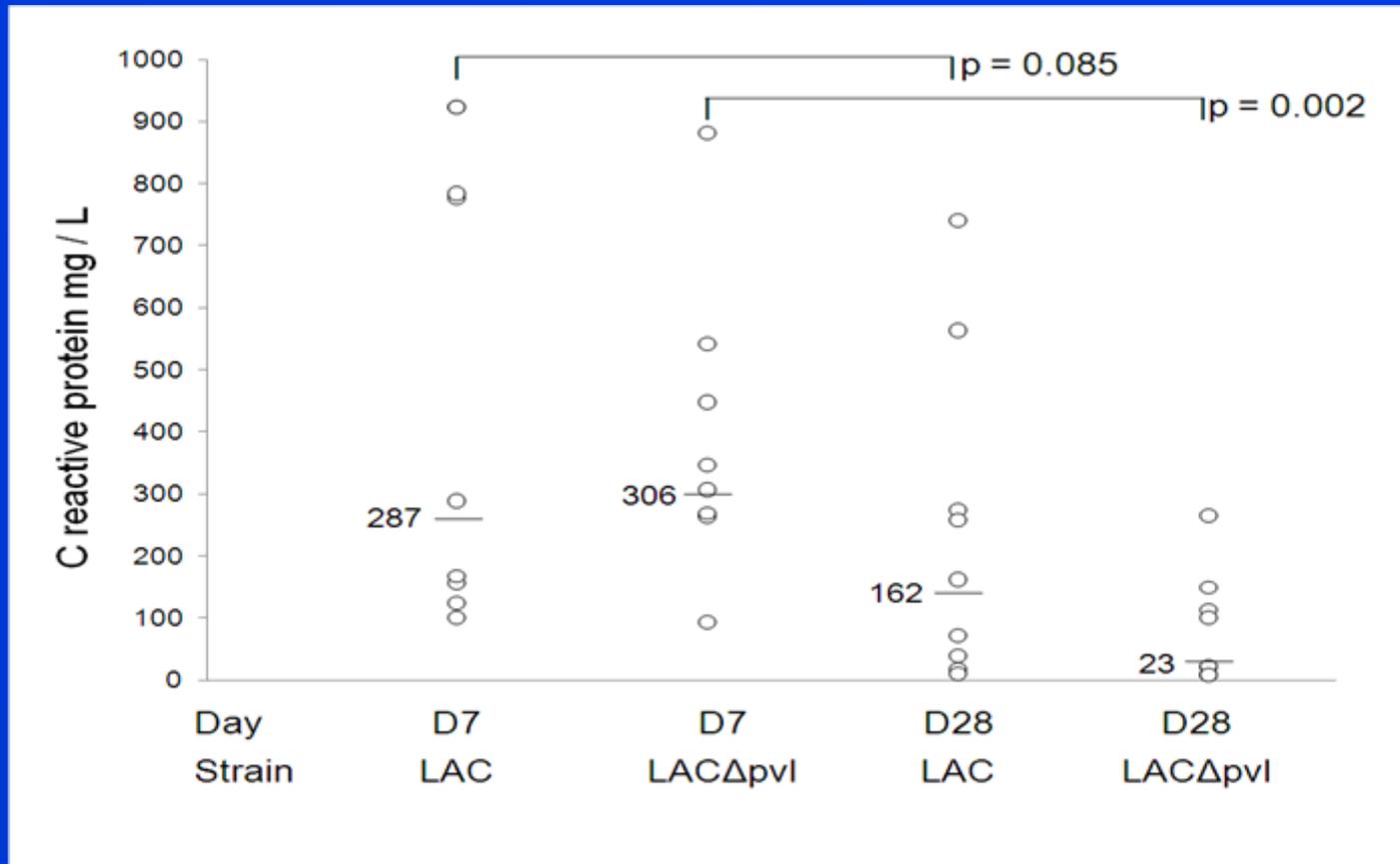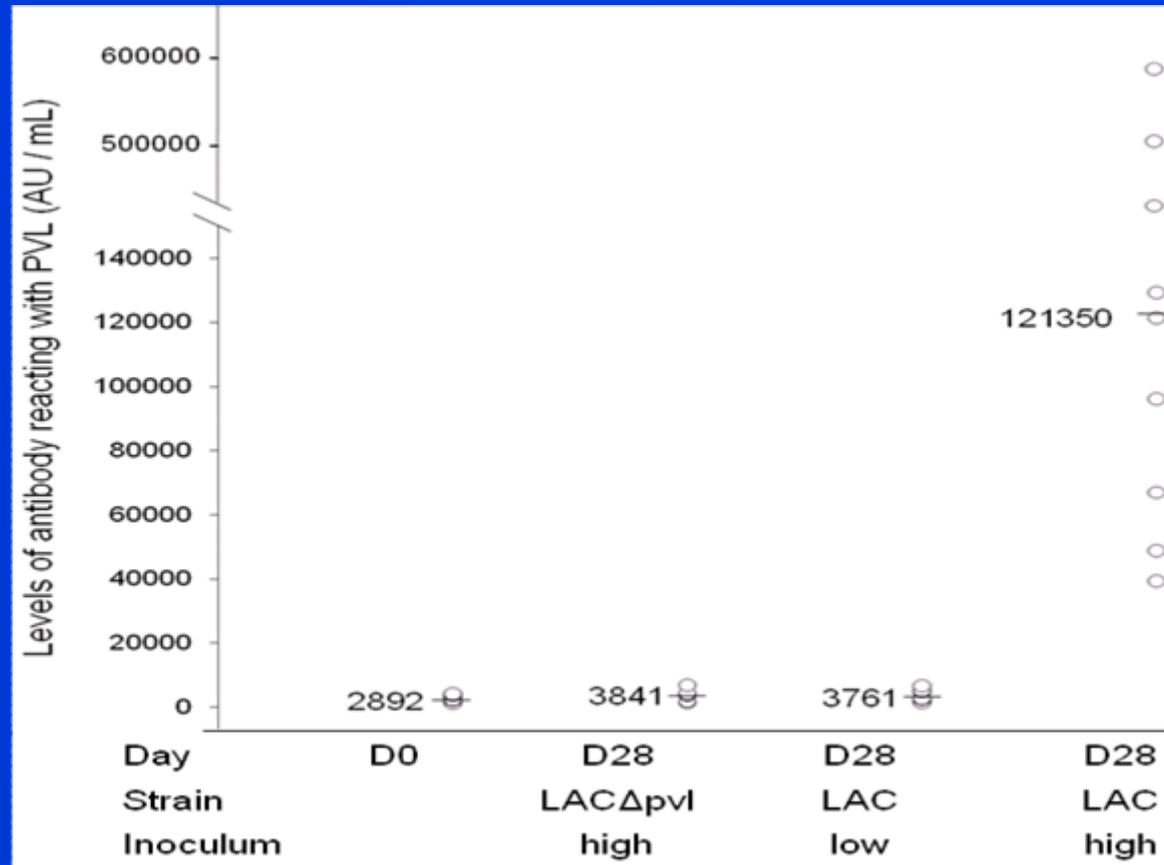

Pyomyositis

Abscess in the joint cavity

Distribution of C-reactive protein (High inoculum)

Distribution of anti-PVL antibody levels in sera

Conclusion

- **Our results showed that PVL could contribute to the pathogenesis of early and late phase of CA-MRSA rabbit osteomyelitis by enabling :**
  - **Better bacterial persistence (low inoculum)**
  - **Local extension during the infection's early phase (high inoculum)**
- **Concordant with clinical studies**

- **Differences with previous experimental explorations of the role of PVL could be explained by**
  - **Location of infection**
  - **The experimental model used: similar to the human situation; allows evaluation at different times post-infection**
  - **Selection of clinically relevant experimental readouts**

- **This model may be suitable to select optimal therapies for PVL⁺CA-MRSA osteomyelitis**