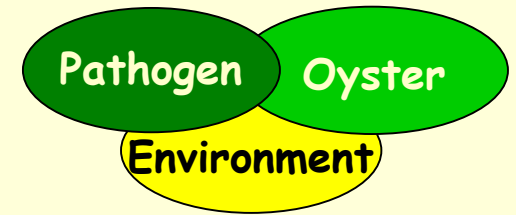


Characterization of *Vibrio* isolated from *Crassostrea gigas* spat suffering from summer mortality outbreaks

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Objectives



- Isolation and identification of *Vibrio* from oysters suffering from summer mortality outbreaks
- Set up of a model of infection trial



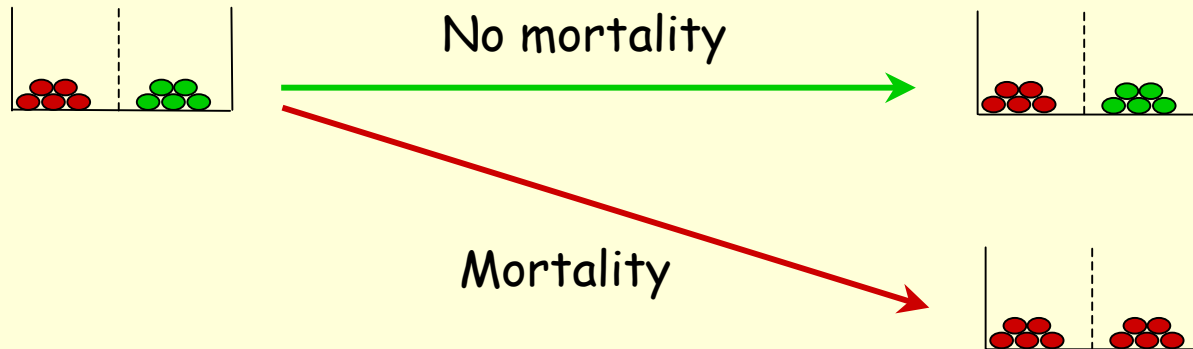
- Selection of strains potentially pathogenic for *C. gigas*



- Disease description

Cohabitation experiments

- Principle



- Results

- Mortality transmission in 11 experiments out of 12
- 125 strains isolated belonging to *Vibrio*:
 - 82 strains isolated from moribund oysters
 - 43 strains isolated from negative controls

Identification of the isolated strains

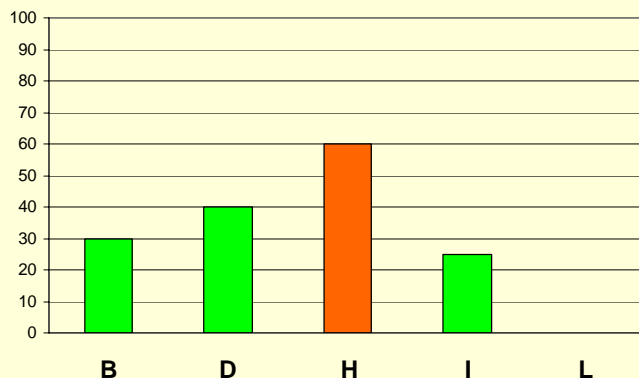
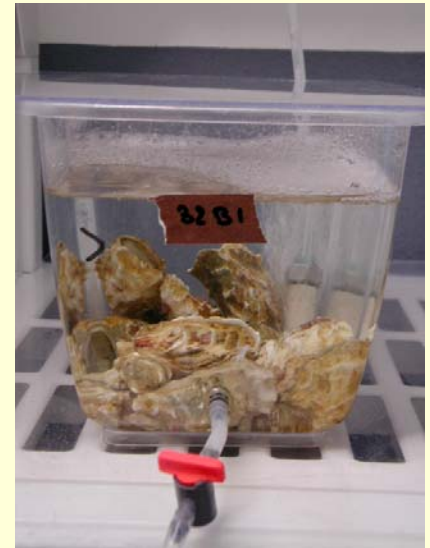
A polyphasic approach

- Techniques
 - Phenotyping and numerical taxonomy
 - Genotyping and phylogenetic analysis : 16S and *gyrB*
- Results
 - **Biodiversity** of the *Vibrio* flora from the **healthy oysters**
 - **Dominance** of the *V. splendidus* group in the **moribund oysters** from all the studied outbreaks leading to a transmission of the mortality

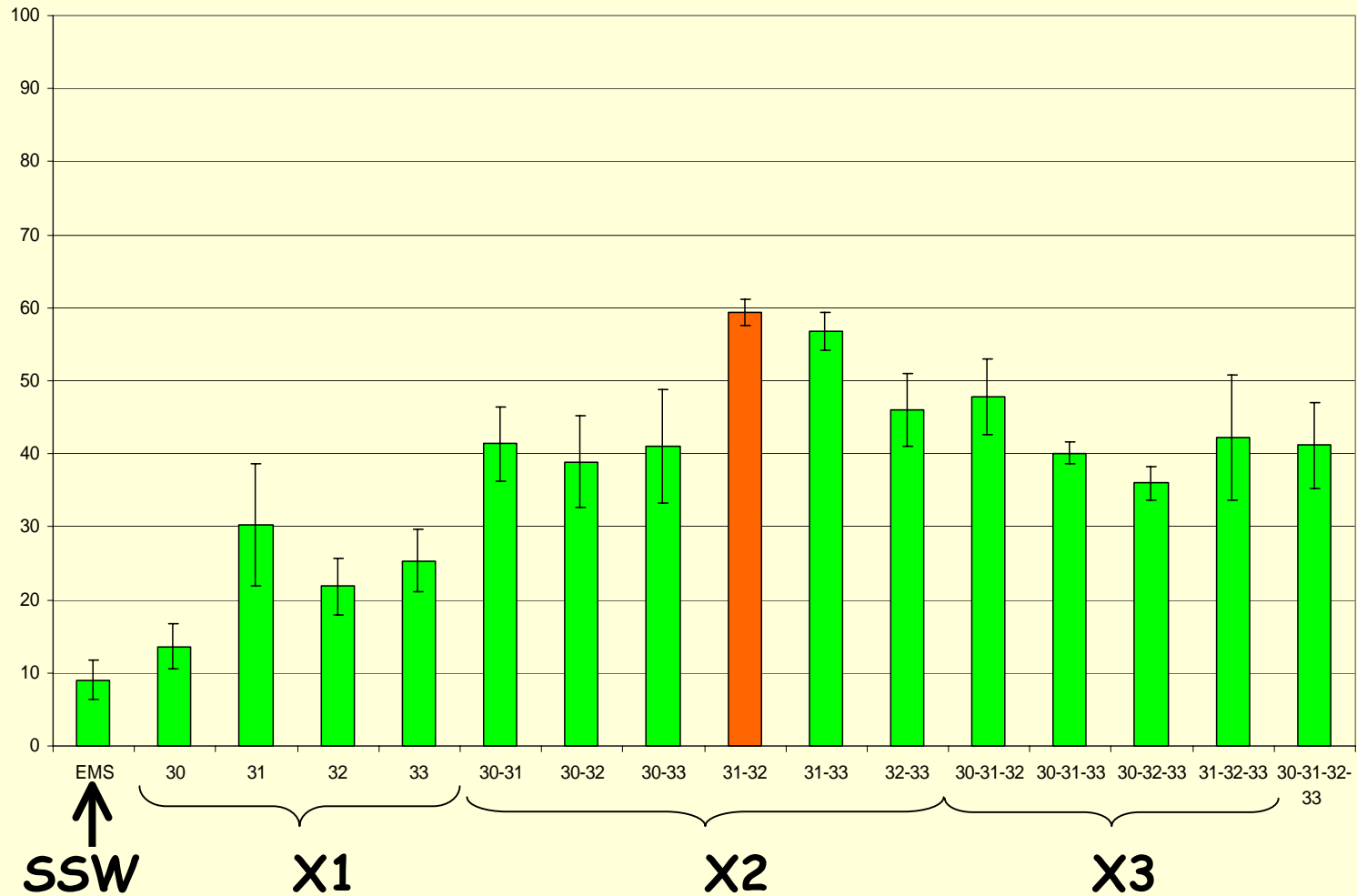
Model of experimental infection

Infection trial : injection in the adductor muscle

- $MgCl_2$ bath
- 3 batches of 30 oysters for each condition
- under static conditions at 20 °C
- Injection in the adductor muscle of 10^7 Bacteria
- Results 3 days post injection
- 1st selection on pools of strains

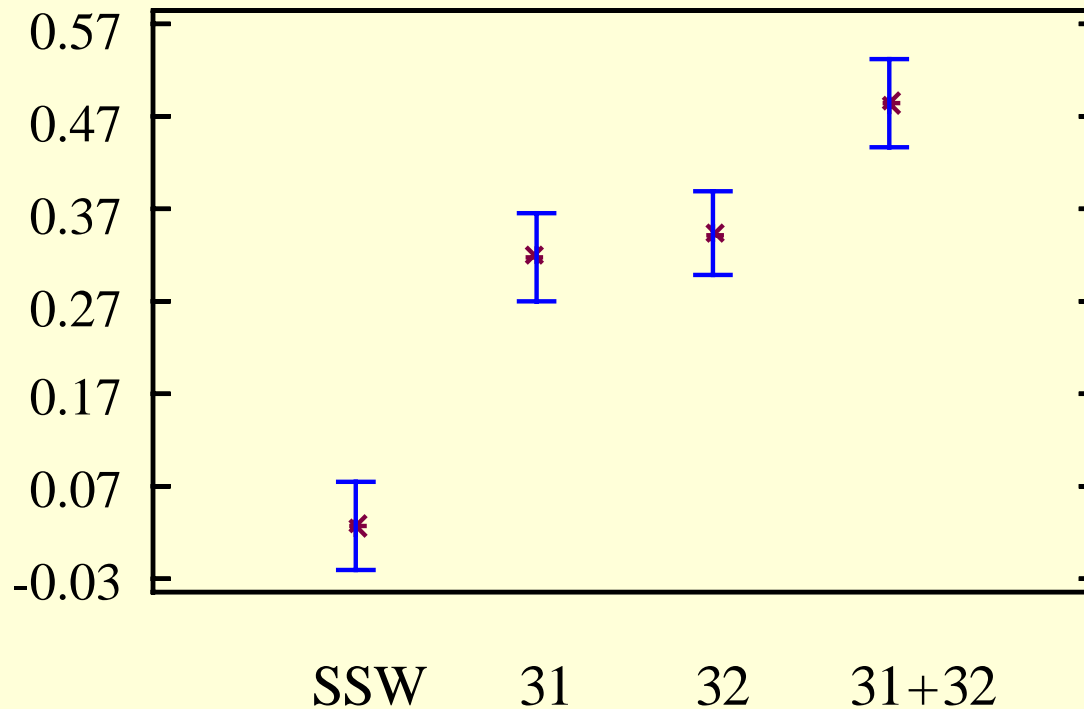


Virulent strains selection : pool H



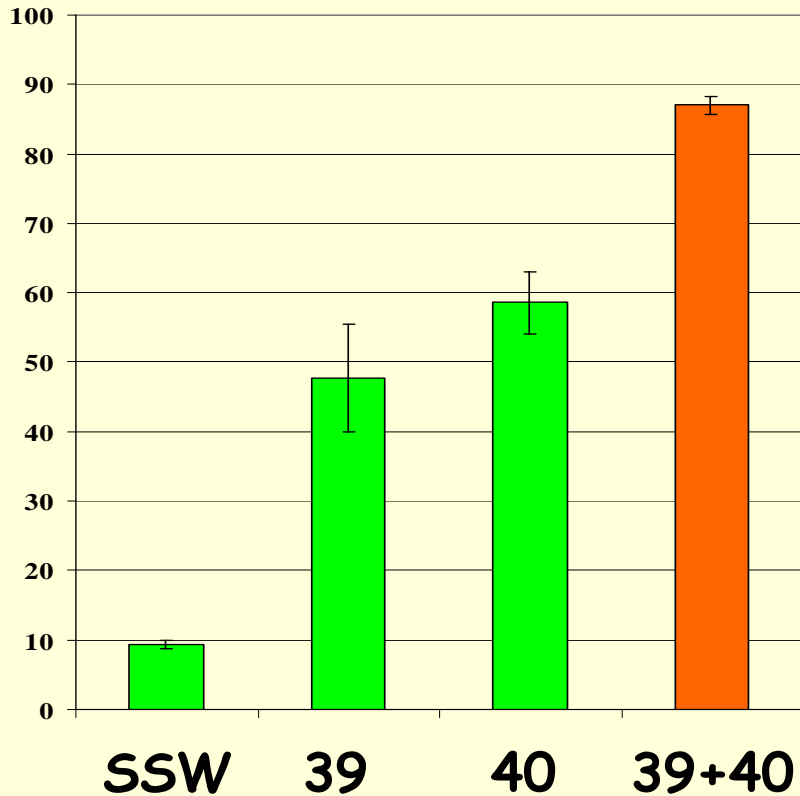
- Highest mortality rate for 31+32

Reproducibility of 31+32

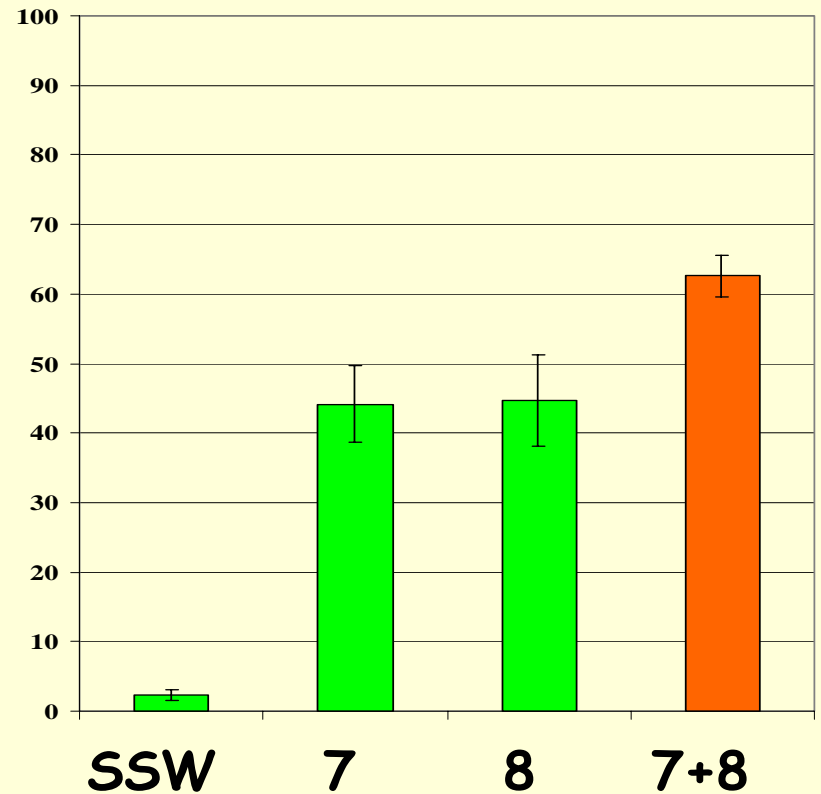


- **Original concept : collaboration of two strains to enhance their virulence**

Collaboration = Epiphenomenon?



Vibrio anguillarum



Vibrio morestus

Conclusions

- Selection of several strains potentially pathogenic for *C. gigas*
- **Original concept : collaboration of two strains to enhance their virulence** : study on the couple 31+32 (*Vibrio lentus*)
- At least two other couples of strains belonging to different *Vibrio* species also show a comparable collaboration

Disease description

Photonic microscopy
Transmission electron microscopy

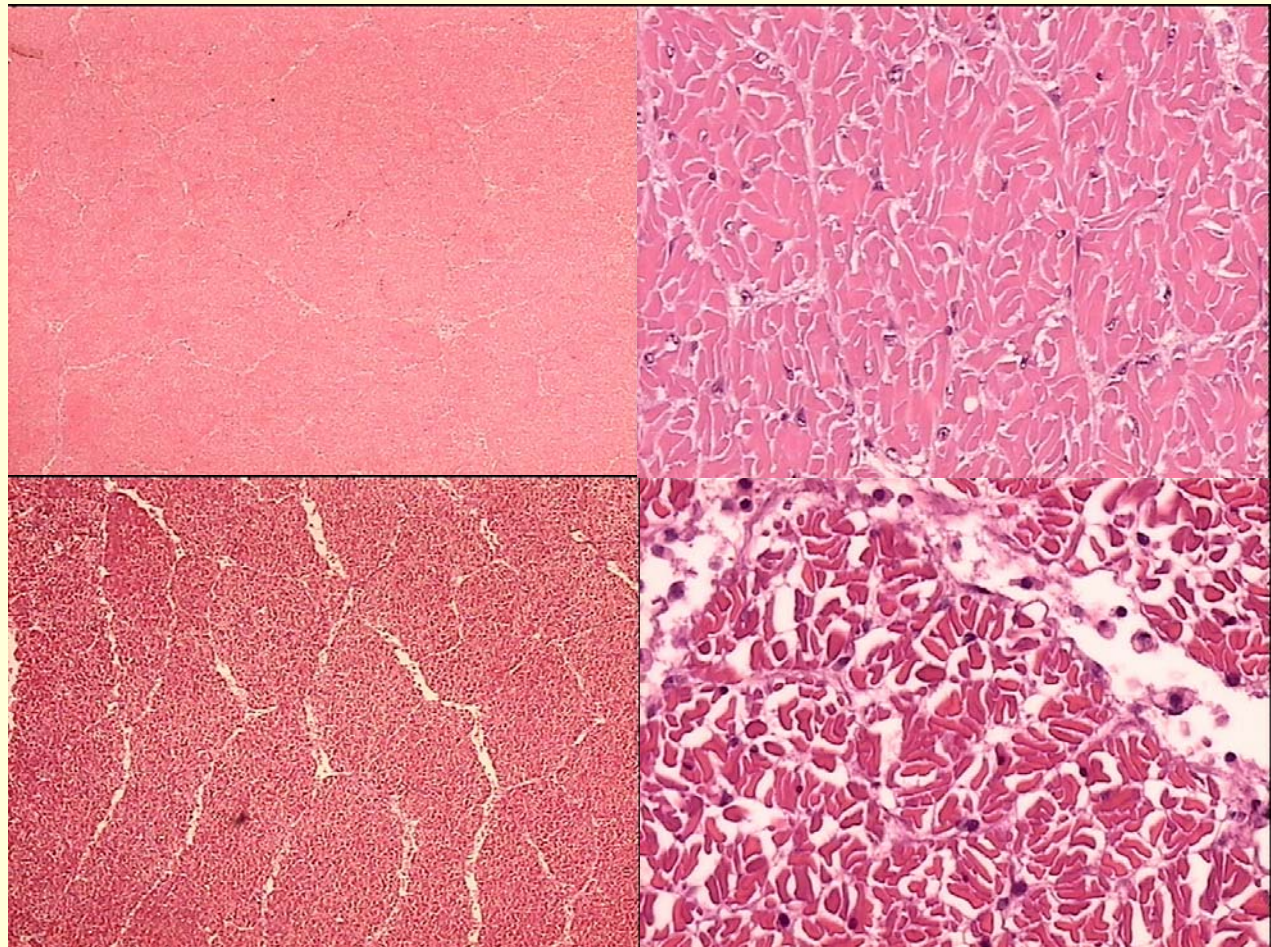
Adductor muscle

- 31+32 : injection in the adductor muscle

x40

x400

Injected
with SSW

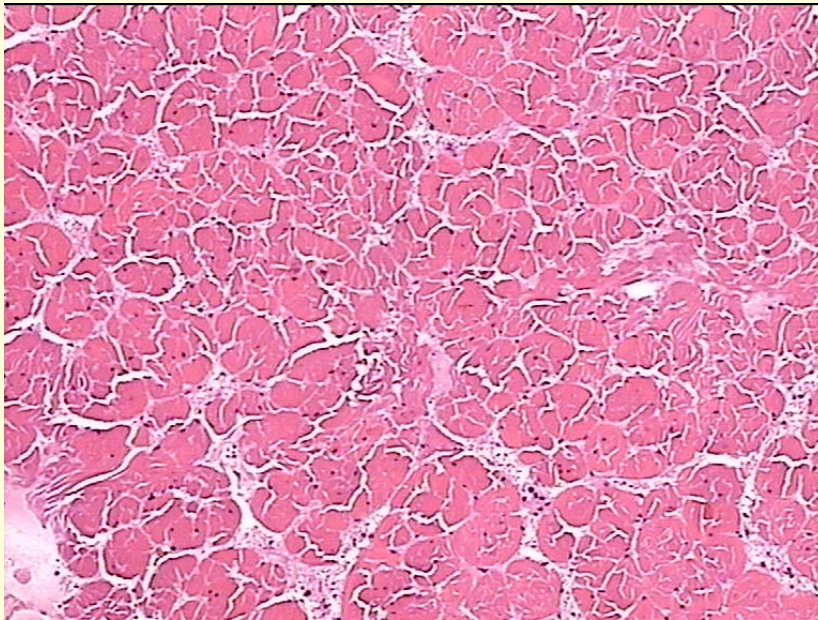


Injected
with 31+32

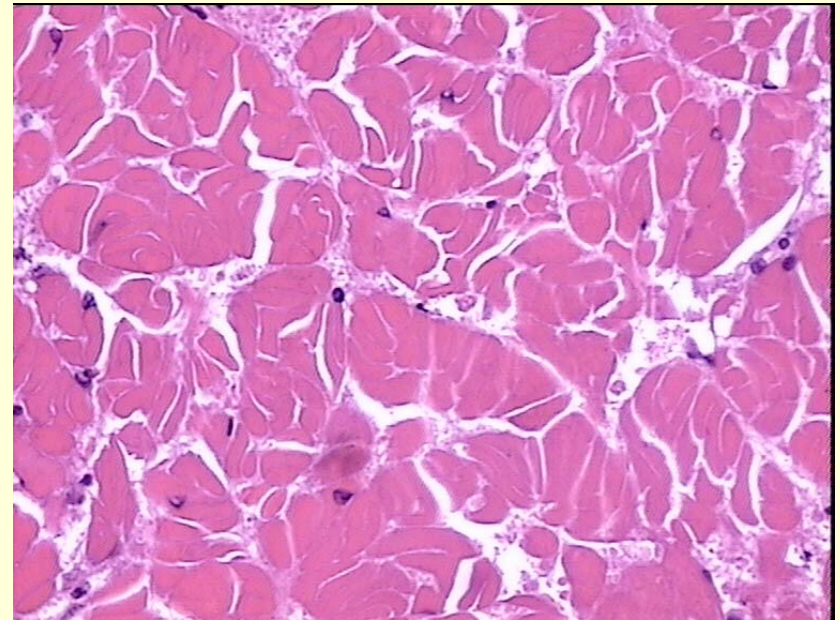
Adductor muscle

- 31+32 : Injection in the palleal cavity

x100

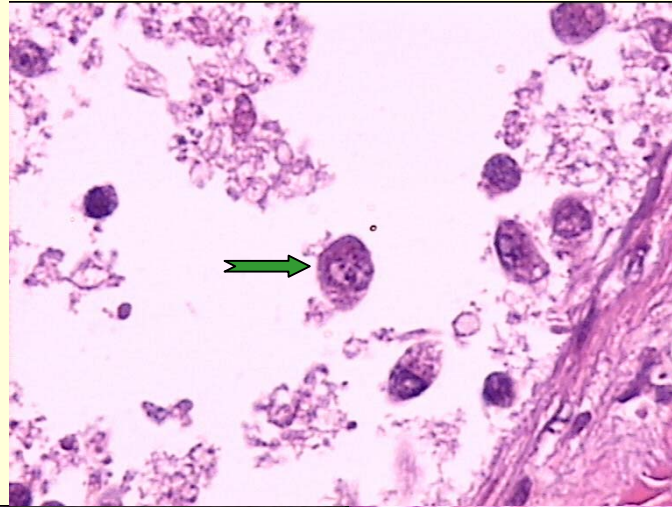


x400

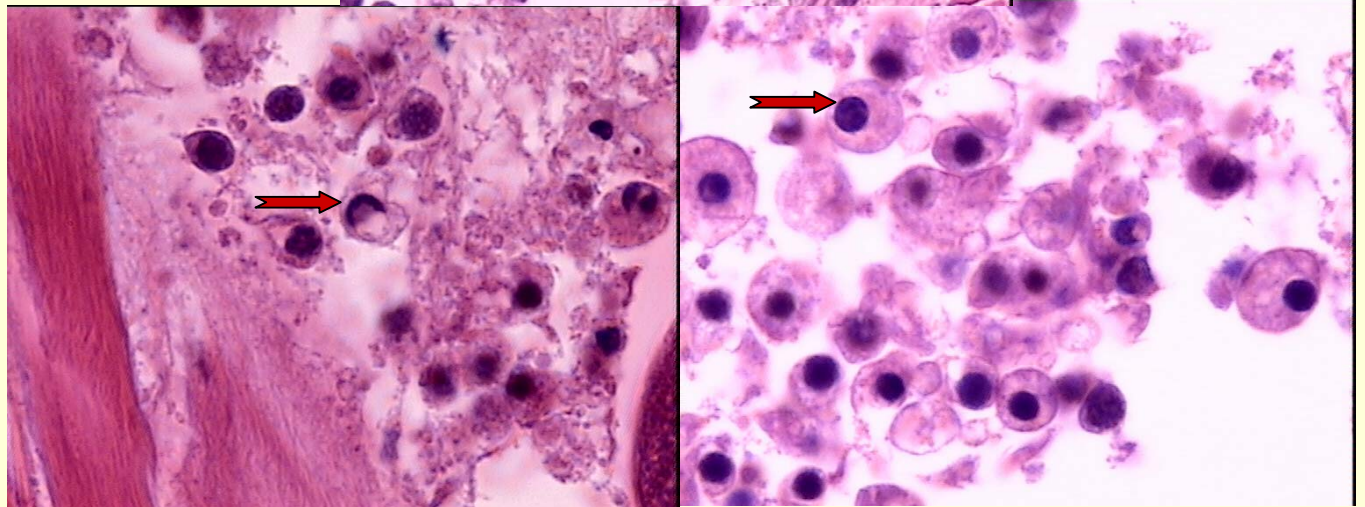


Circulating cells

Injected
with SSW

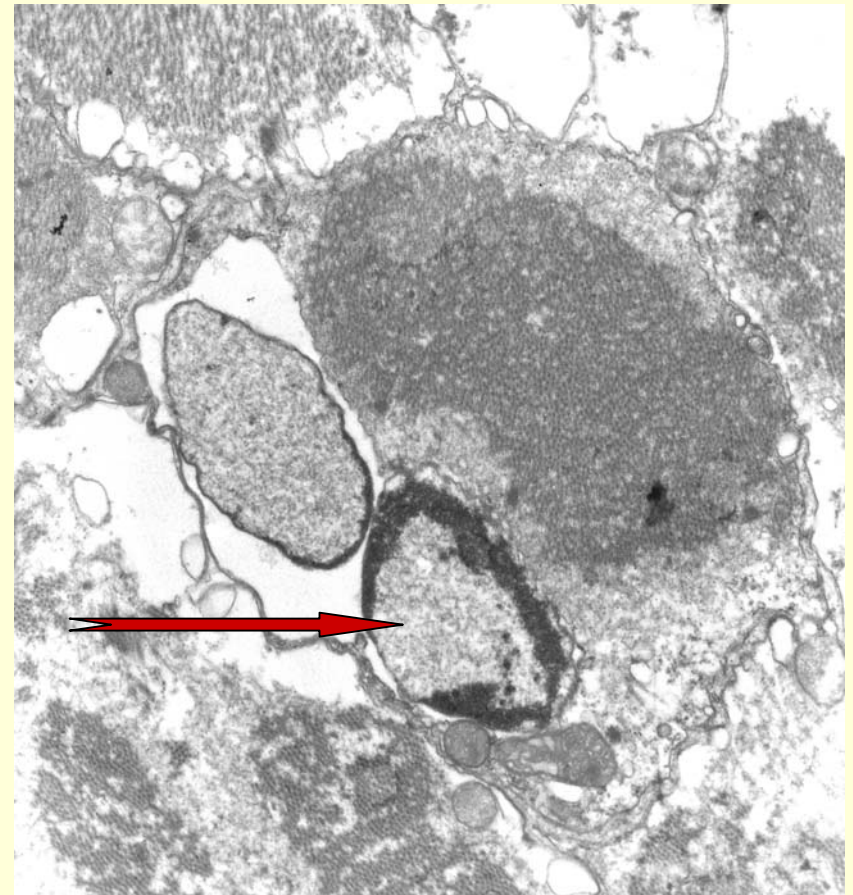
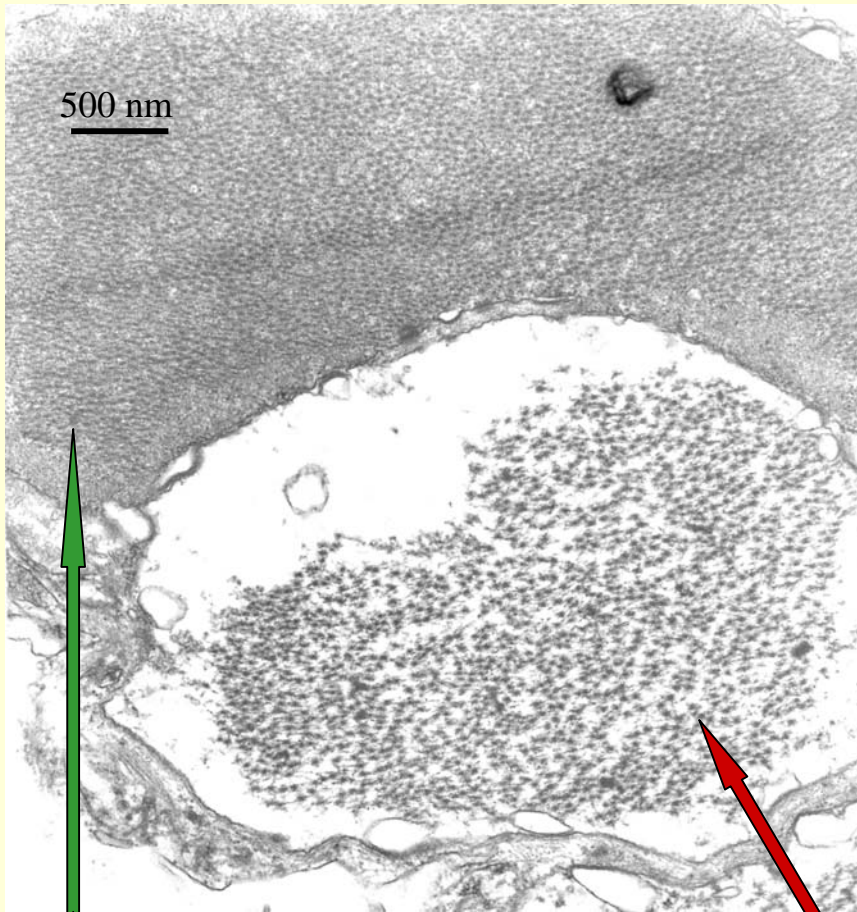


Injected
with 31+32



Tissue alterations

- Transmission electron microscopy

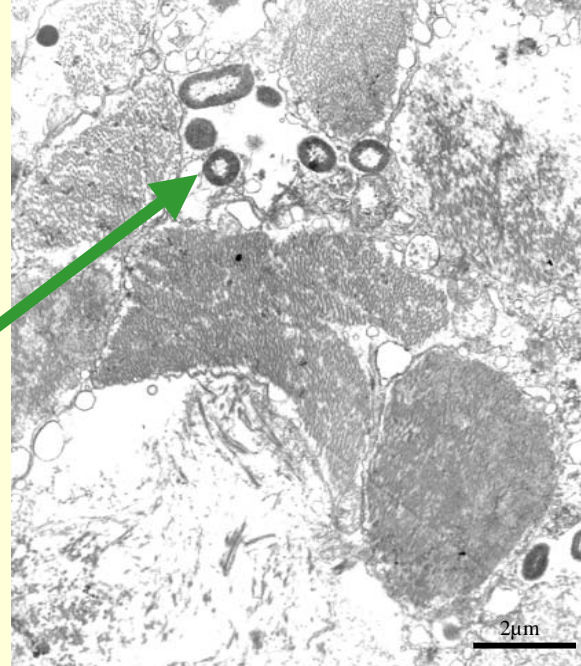
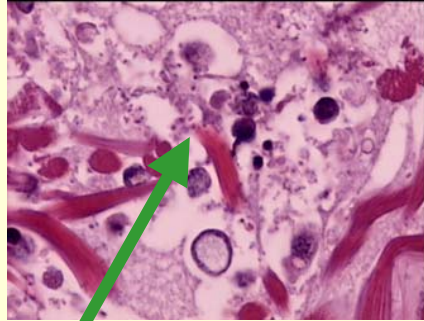
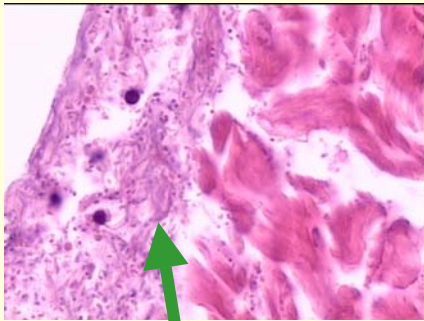


Healthy muscular fiber

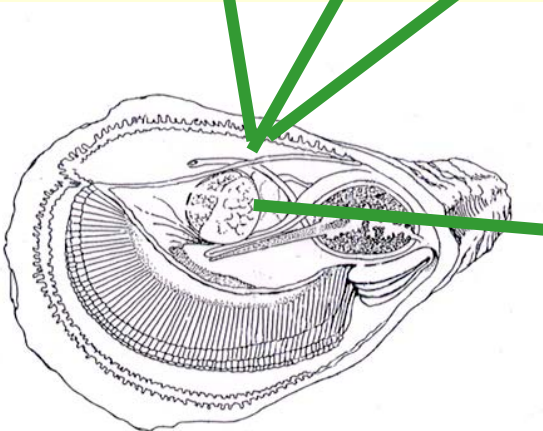
Degenerating muscular fiber

Bacteria localizations

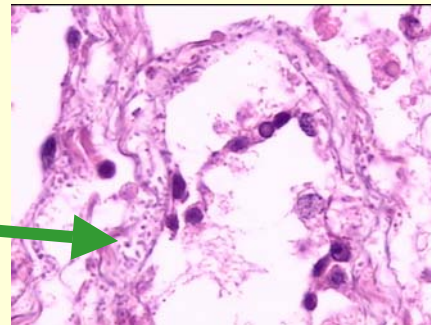
- 31+32 : injection in the adductor muscle



Around the muscle

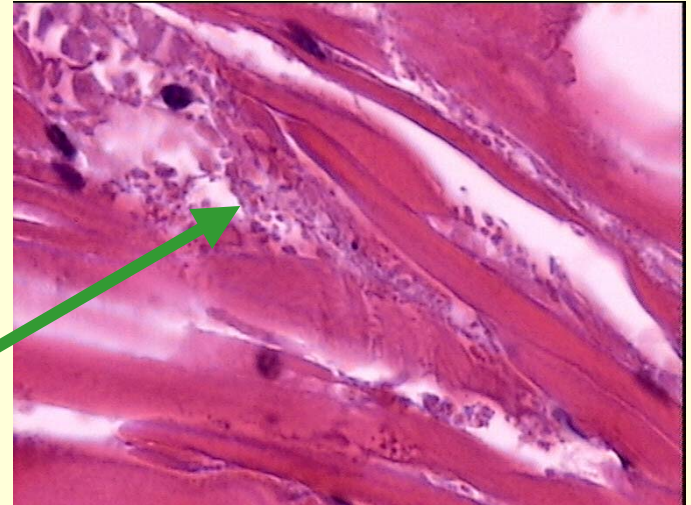
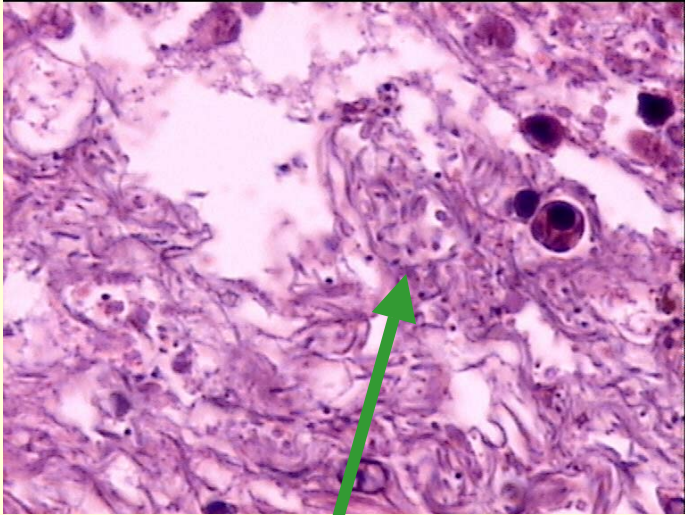


In the kidney

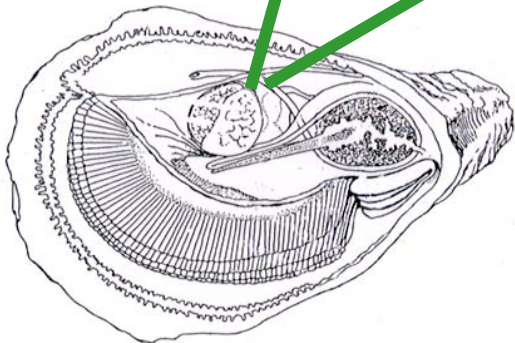


Bacteria localizations

- 31+32 : injection in the palleal cavity



Around the muscle



Conclusions

- Important destruction of the muscular tissue
- Alteration of the nuclei of circulating cells and muscle fibers
- Bacteria localization around the muscle (conjunctive tissue and muscle fibers)
- **Identical lesions and bacteria localization after injection in the adductor muscle or in the palleal cavity**

Perspectives

- Development of diagnostic tools specific for 31 or 32
- Studies on the other groups of virulent strains (*V. anguillarum* and *V. "morestus"*) to determine the different mechanisms of virulence
- *In vitro* tests to evaluate the virulence of these bacteria
- *Ex vivo* models :
 - Nuclei alterations (necrosis or apoptosis?)
 - Hemocytes reaction and genes expression