

# Tissue lesions induced by OsHV-1 $\mu$ Var and their evolution over time

B. Chollet, B. Morga, L. Dégremont,  
M. Noyer, C. Dubreuil, D. Serpin,  
I. Arzul, C. Garcia

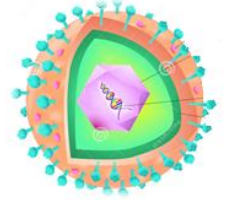


LGPM-Ifremer La Tremblade



## Context

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Detection of OsHV-1  $\mu$ Var **by Real-Time PCR** in different organs during experimental infections (Schikorski *et al.* 2011, Segarra *et al.* 2016)

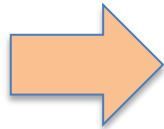
⇒ gills and mantle: potential targets of the virus

Description of lesions induced by OsHV-1 from animals collected during **mortality events**

⇒ tissue necrosis and/or hemocyte infiltrations associated with nuclear abnormalities (Hine *et al.* 1992, Renault *et al.* 1994 and 2001)

Few information regarding OsHV-1  $\mu$ Var infection in histology

## Objectives

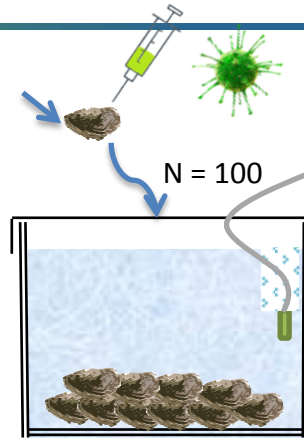


### Description of

- lesions associated with OsHV-1  $\mu$ Var
- OsHV-1  $\mu$ Var distribution in oyster tissues during a controlled infection

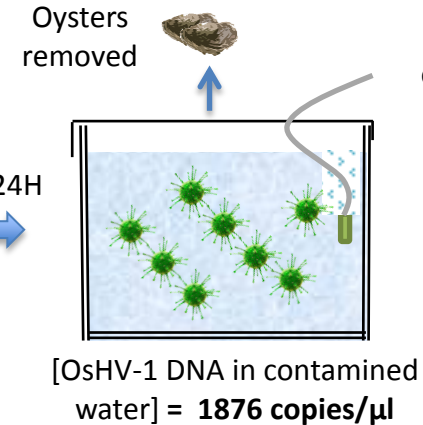
## Experimental design

1 - Oyster injected with  
100 $\mu$ L  
OsHV-1  $\mu$ Var suspension



After 24H

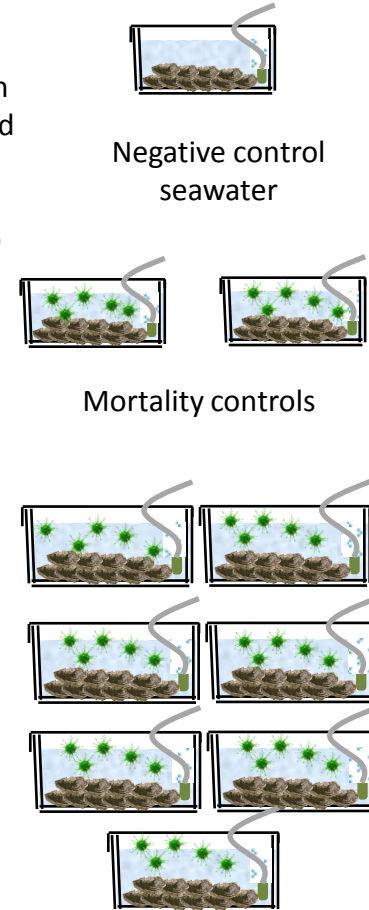
Oysters removed



[OsHV-1 DNA in contaminated water] = 1876 copies/ $\mu$ L


The diagram shows a hand removing an oyster from a tank. The water in the tank is now filled with green virus particles. A blue arrow points from the first tank to this one.

2 - Distribution  
of contaminated  
water and  
oysters  
(30/aquarium)



3 - sampling : 1 aquarium  
(30 oysters) twice a day over 4 days

### Analyses performed



Real-Time PCR TaqMan OsHV-1  
Histology  
*In situ* hybridisation  
TEM

A blue arrow points from the sampling step to this analysis box.

### Oysters

9 months-old oyster spat produced in Ifremer facilities

(wild *C. gigas* crosses)

- No mortality during the production cycle
- Before the experiment, 30 oysters analysed
  - No pathogen detected (bacteriology, histology, qPCR OsHV-1)

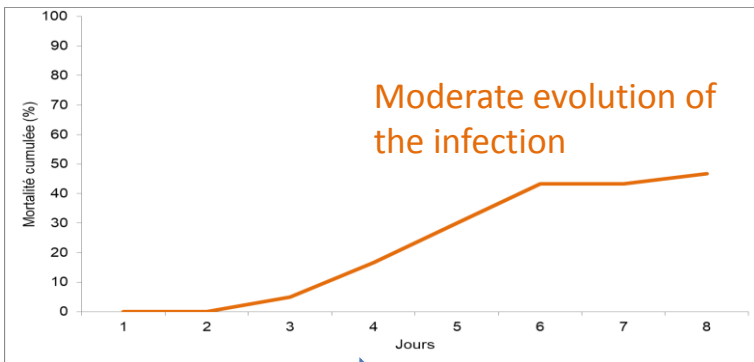
## Monitoring of OsHV-1 infection

### Negative control

- ⇒ No mortality observed
- ⇒ No detection of OsHV-1 or other pathogen

### Mortality control

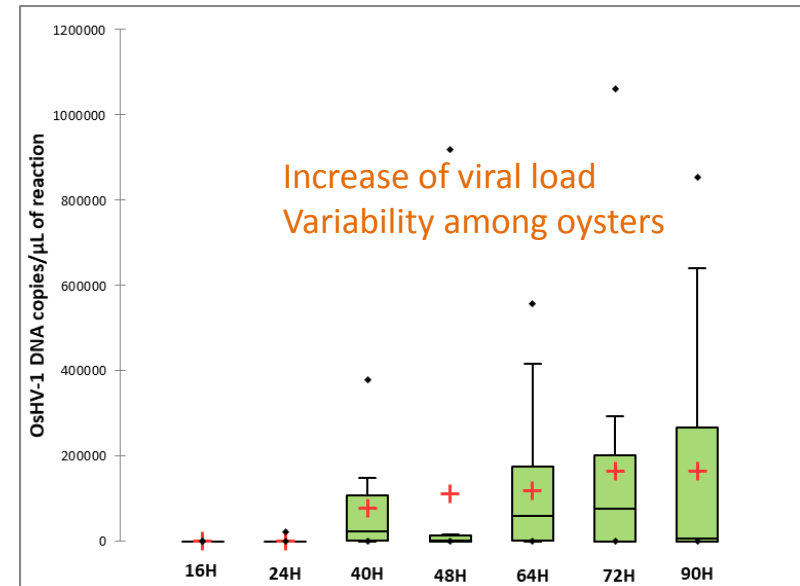
- ⇒ First mortalities occurred at day 3
- ⇒ 47% of mortality at the end of experiment (day 8)



Sampling period  
(2 times/day)

### Monitoring of OsHV-1 infection

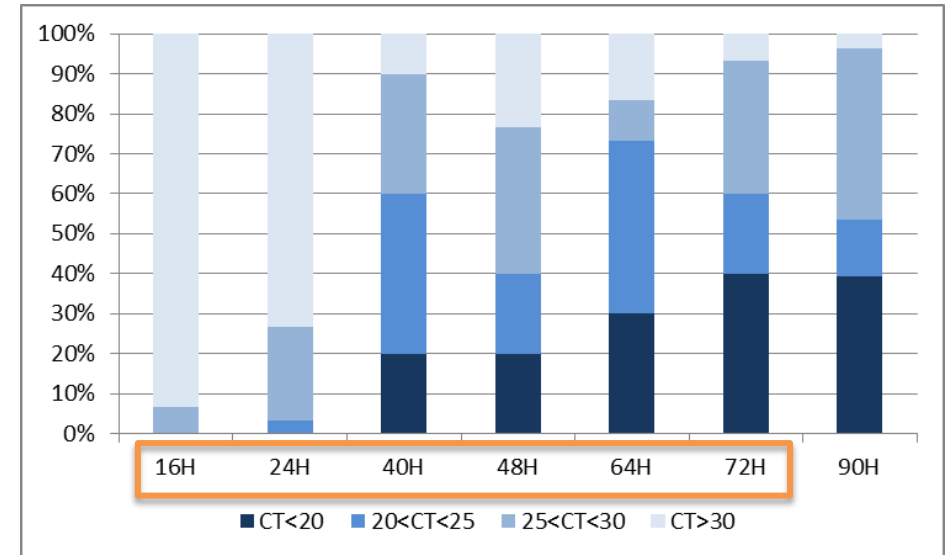
208 individuals tested by Real-Time PCR over 4 days (7 sampling times)



## Monitoring of OsHV-1 infection

### Classification of the 208 individuals sampled according to the viral load in 4 categories

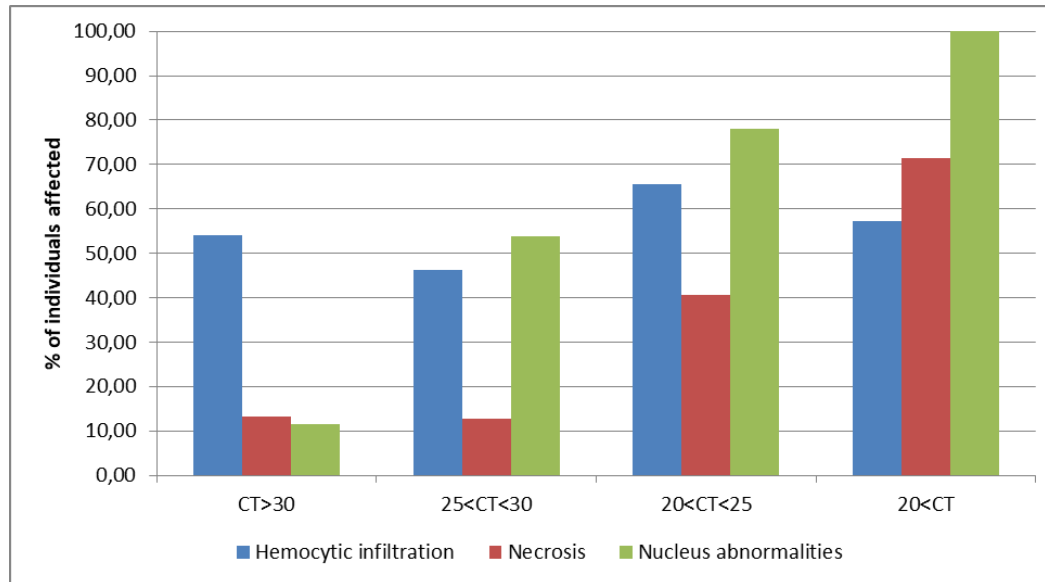
CT value	Number of viral DNA copies / $\mu\text{l}$ of réaction	Number of individuals
CT>30	Less than 500	68
25<CT<30	Between 500 and 5 000	54
20<CT<25	Between 5 000 and $10^5$	42
CT<20	$>10^5$	44



=> Focus on samples collected between 16 and 72 h

## Lesion evolution

Main tissular lesions : hemocyte infiltration, nuclear abnormalities and necrosis



**Hemocytic infiltration** was observed all along the infection

**Necrosis** and **nucleus abnormalities** increased with the development of the infection

## Lesion evolution: Hemocytic infiltration

Mostly focal infiltration, rarely systemic even in advanced infection

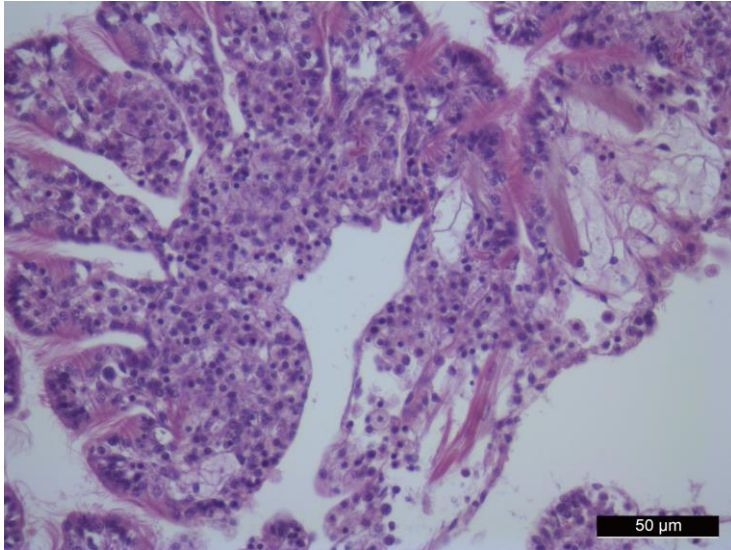
Mainly observed in gills, mantle and digestive gland



Histological section of mantle  
Beginning of OsHV-1 infection

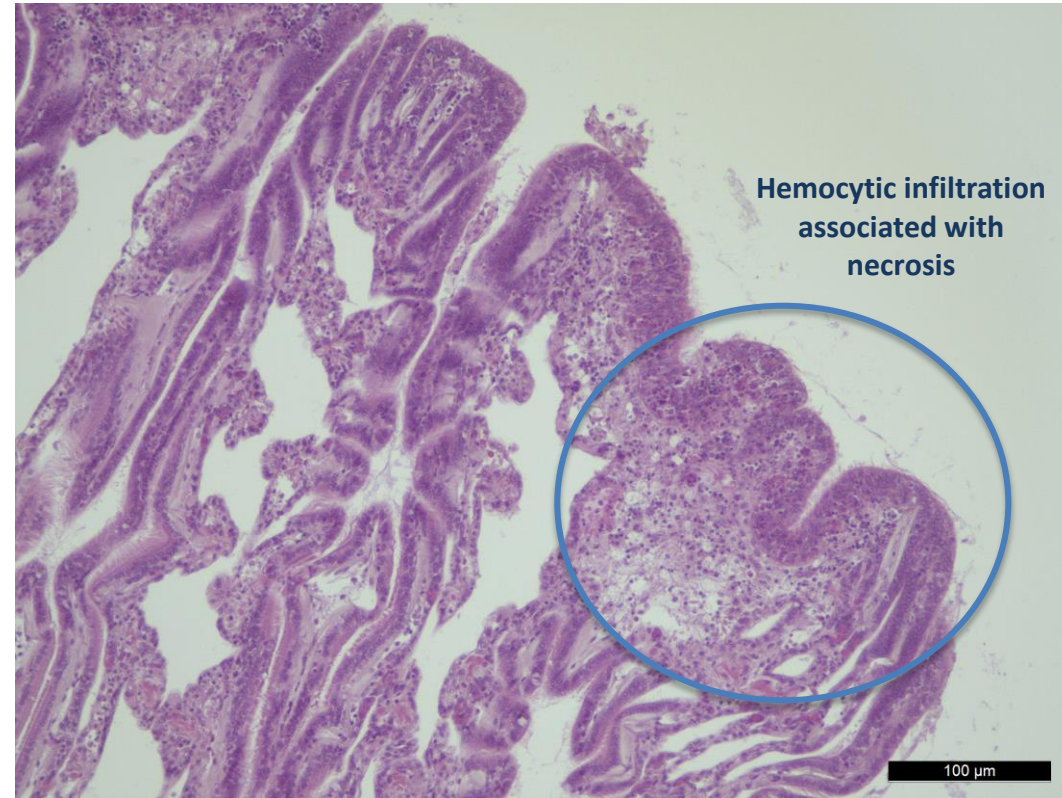


## Lesion evolution: Hemocytic infiltration



**Hemocytic infiltration  
associated with  
necrosis**

Histological section of gills  
Advanced OsHV-1 infection



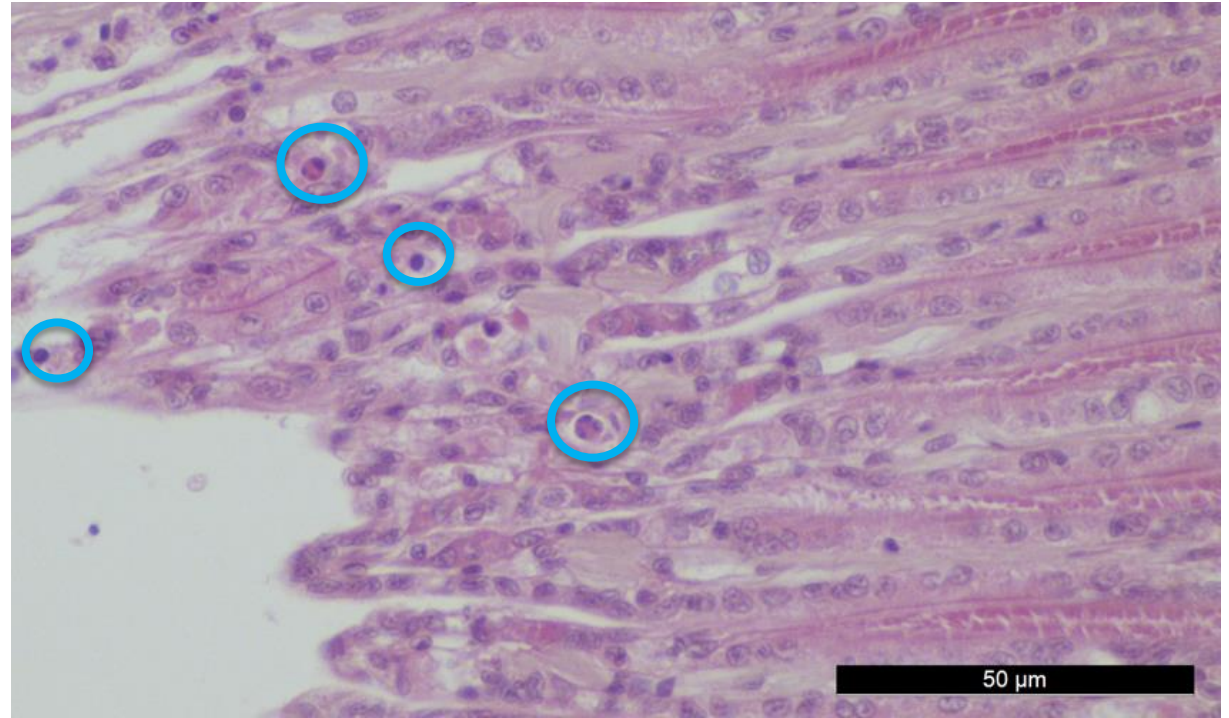
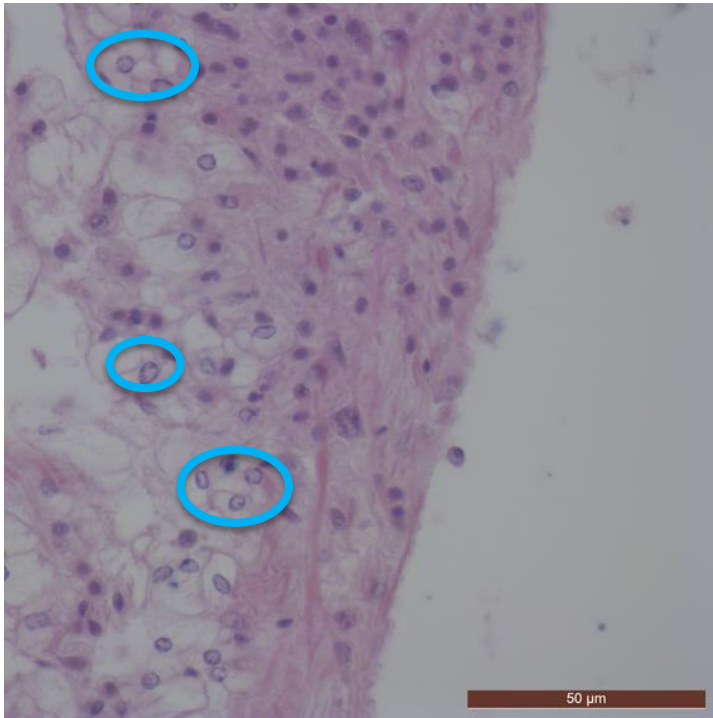
**Hemocytic infiltration  
associated with  
necrosis**

In advanced infection, haemocytic infiltration is often associated with necrosis



## Lesion evolution: Nuclear abnormalities

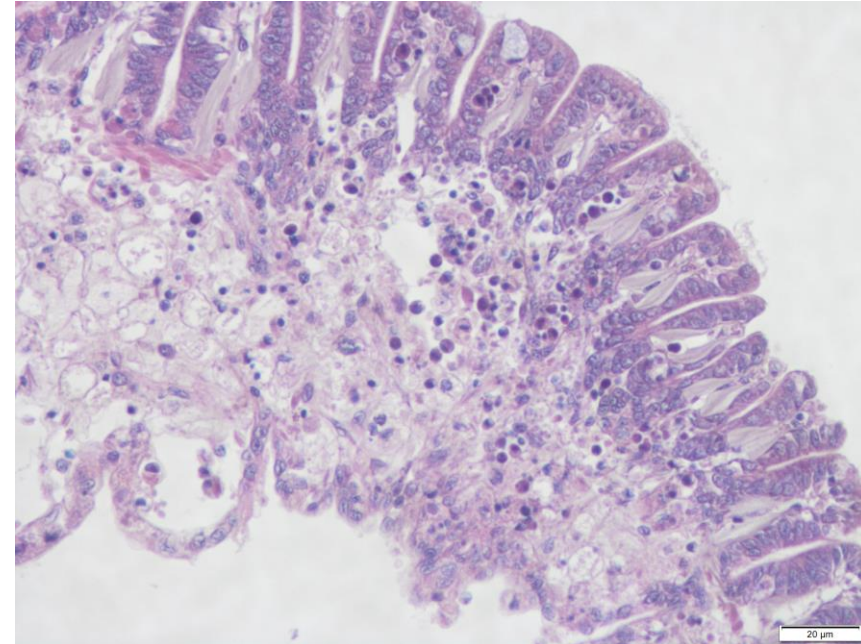
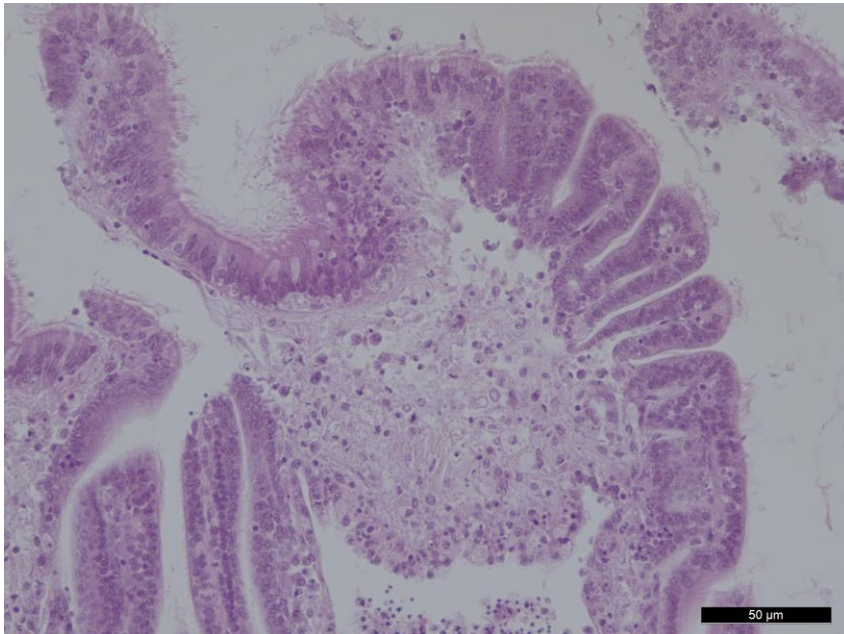
3 main nuclear abnormalities : margined chromatin, pyknotic nucleus or nucleus fragmentation  
mainly located in gills and mantle at the beginning of infection



## Lesion evolution: Necrosis

During the acute phase of the infection:

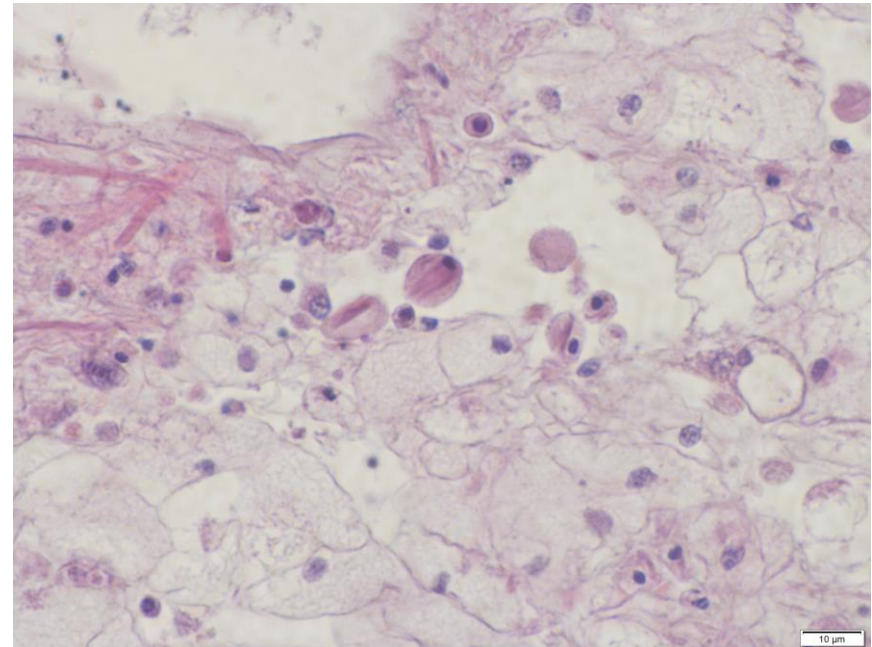
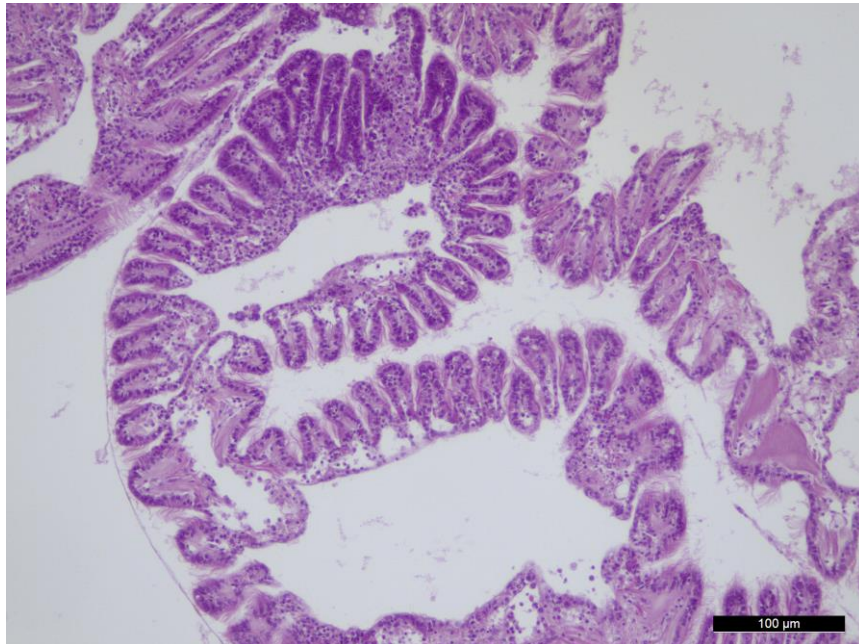
- necrosis lesions extend to all organs with an increase in their intensity
- increase of nuclear abnormalities in the connective tissues including in heart and nervous tissues
- migration of abnormal cells in epithelia



## Lesion evolution: Necrosis

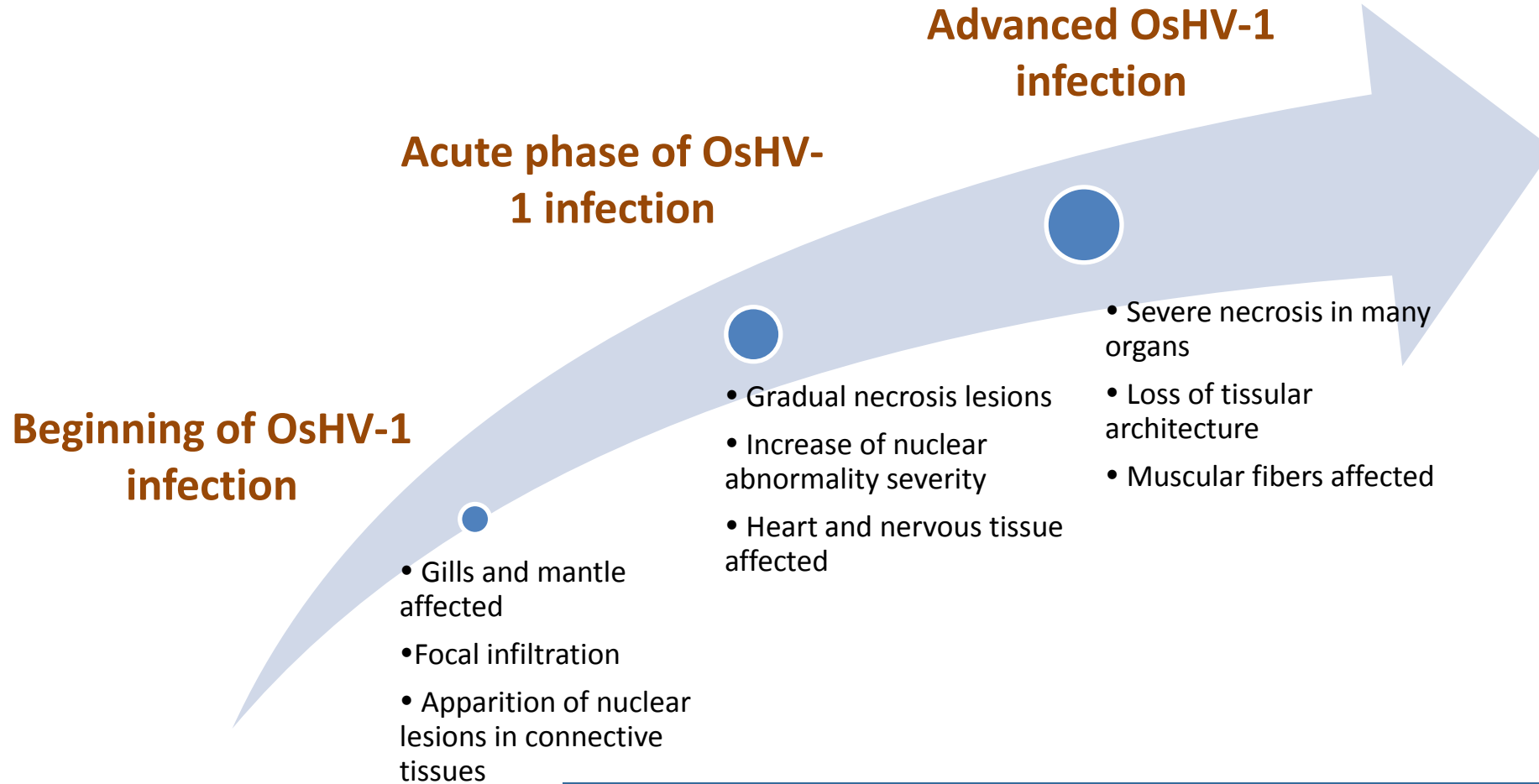
In more advanced infection:

- observation of pycnotic nuclei and nucleus fragmentation associated with necrosis lesions
- loss of tissular architecture
- lesions of muscular fibers



## Dynamics of lesion appearance

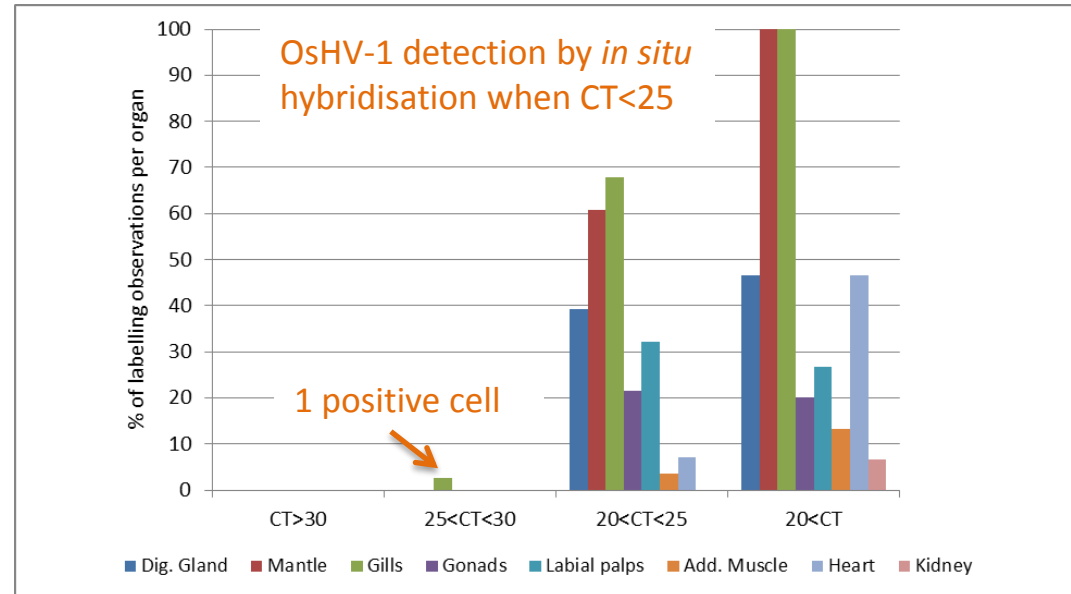
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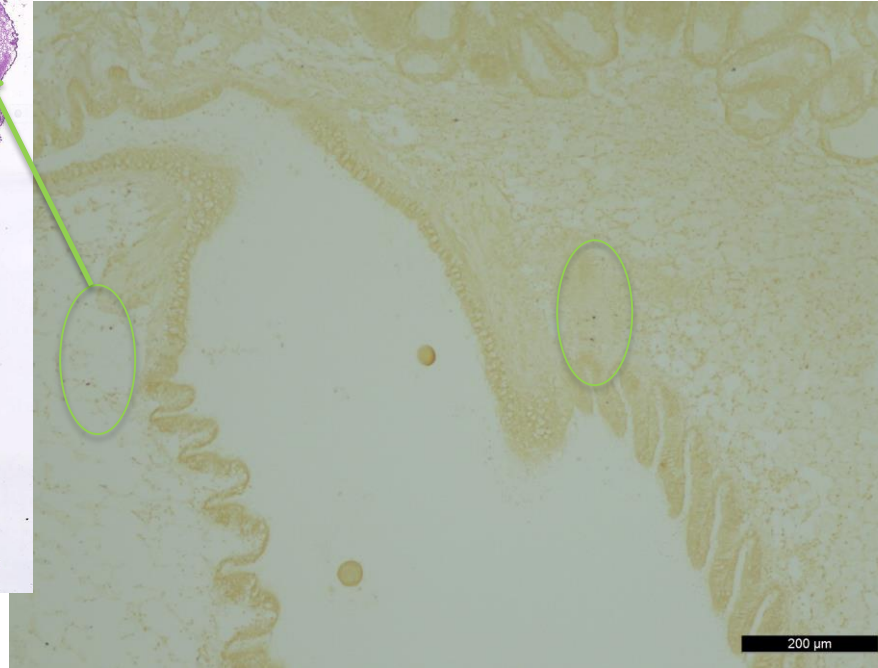
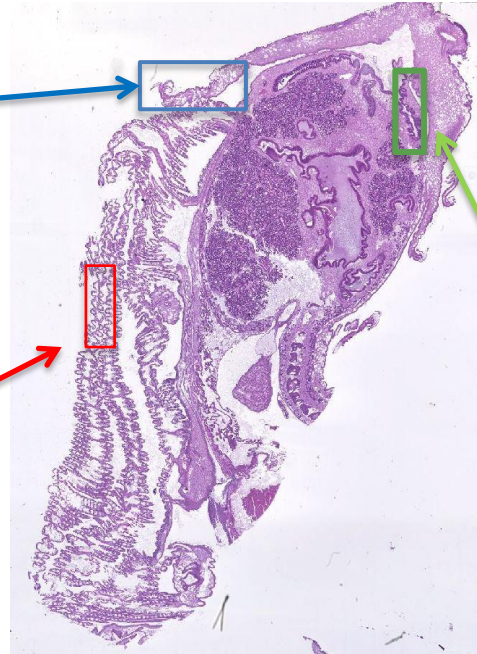
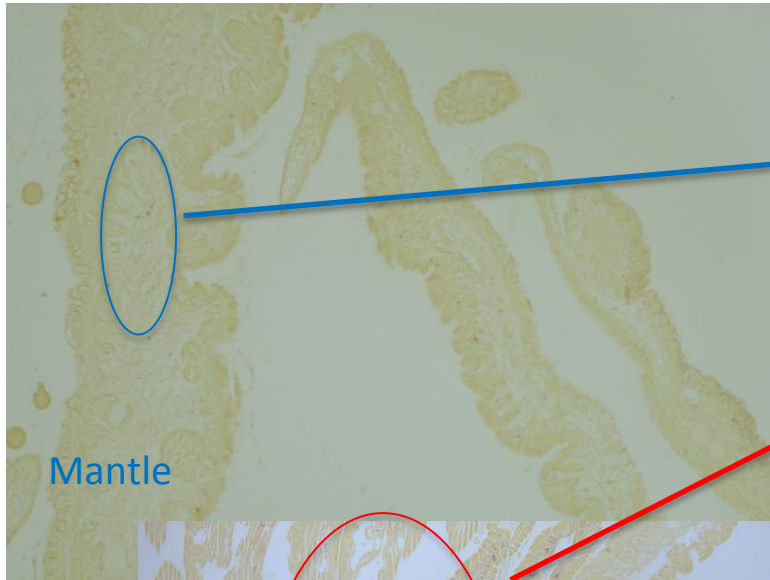
## OsHV-1 development

### *In situ* hybridization :

- First detection of OsHV-1 DNA in gills
- Gills and mantle are the main organs where the OsHV-1 infection develops
- In advanced infection, OsHV-1 DNA detected in all the organs
- OsHV-1 DNA detected in different cells: connective tissue cells, hemocyte-like cells, ...
- No detection of OsHV-1 in epithelial cells



# OsHV-1 development 20<CT<25

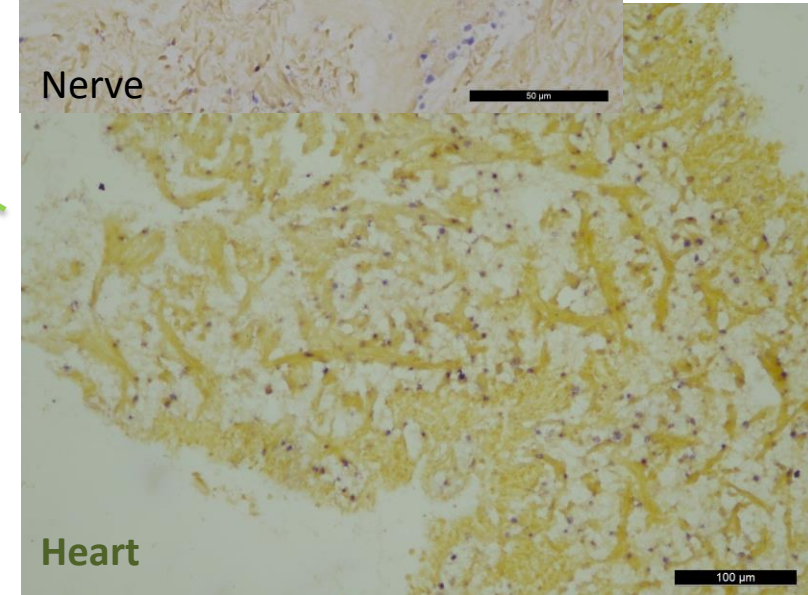
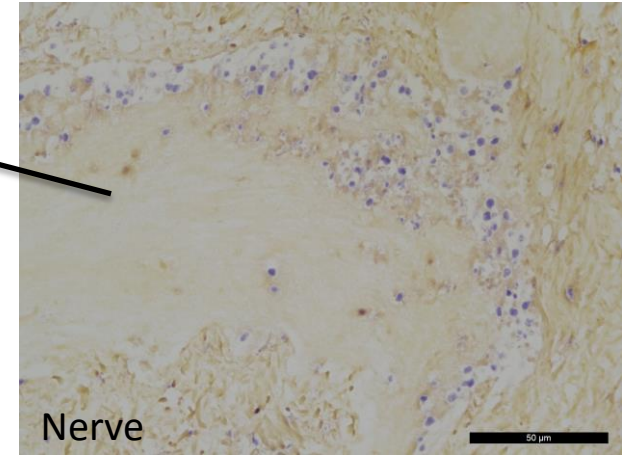
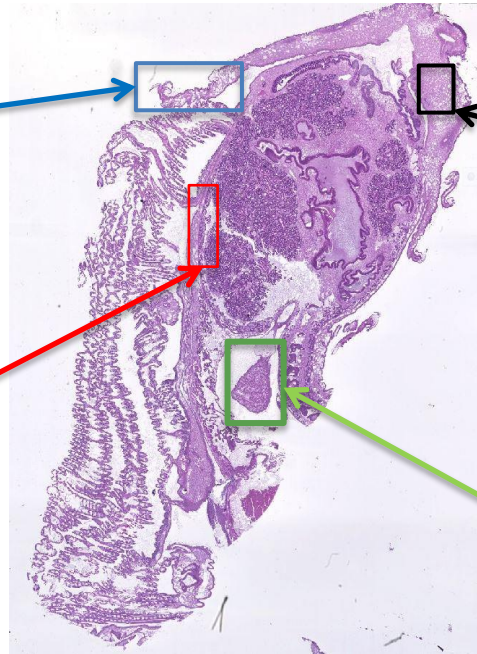
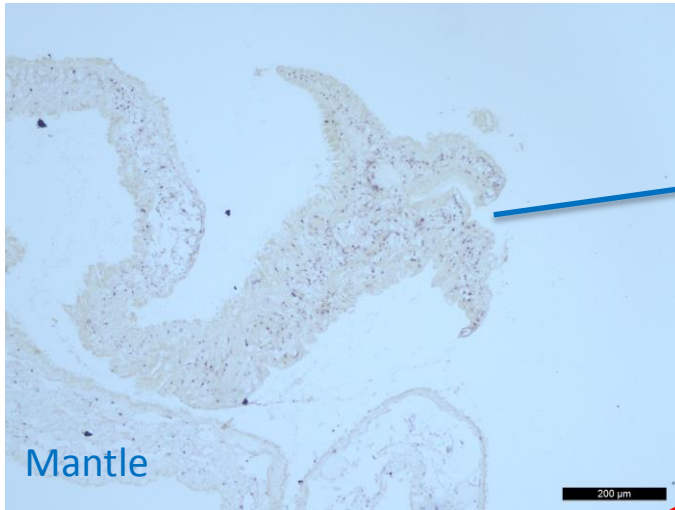


Labial palps

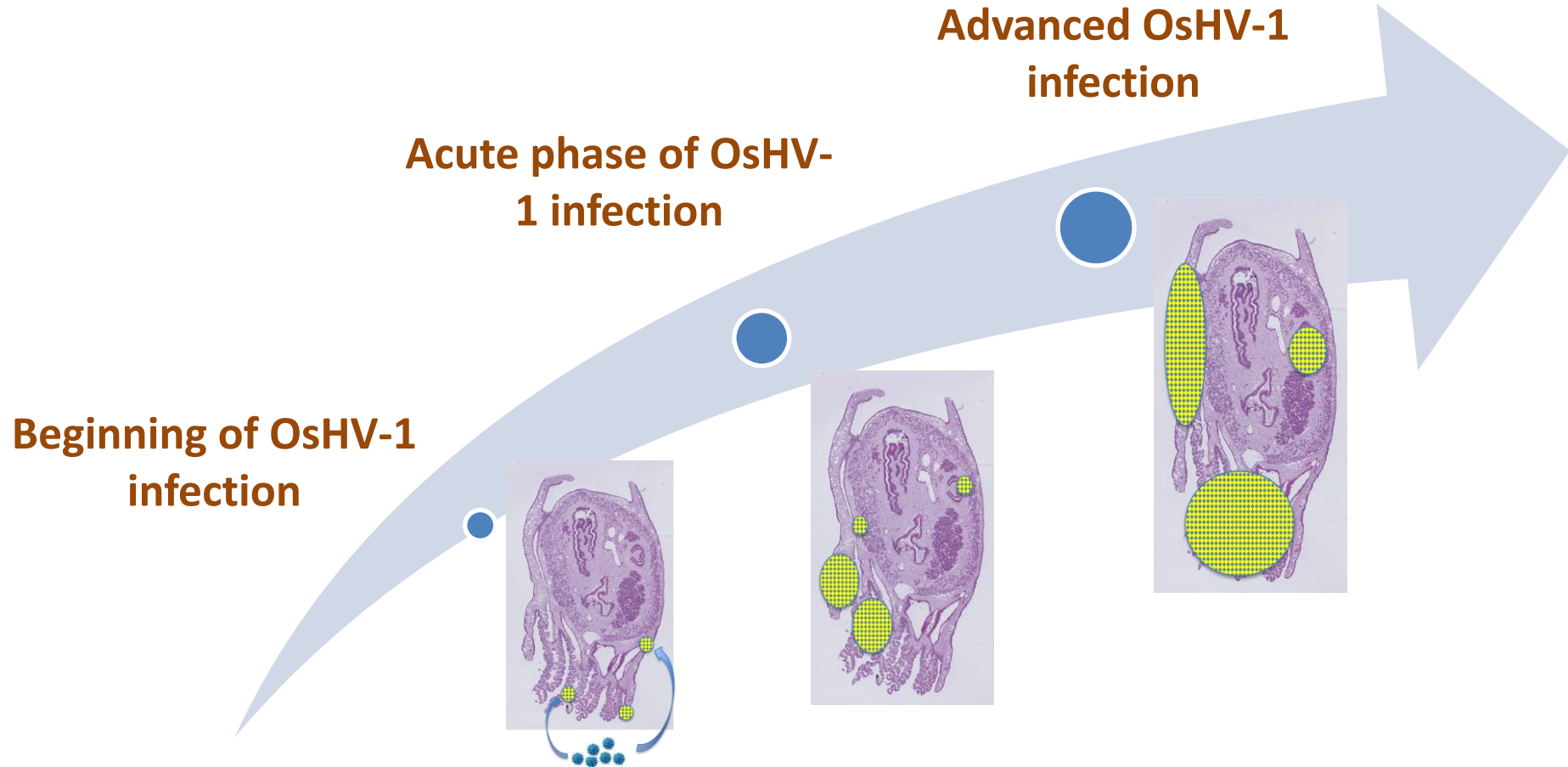


Gills

# OsHV-1 development 20<CT



# OsHV-1 development





## Conclusions and perspectives

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- ✓ OsHV-1  $\mu$ Var first infects gills and mantle, and, then other organs
- ✓ At the tissular level, OsHV1  $\mu$ var infects connective tissues contributing to a disruption of tissular architecture
- ✓ OsHV-1  $\mu$ Var preferentially infects connective tissue cells but also cells looking like hemocytes and possibly nervous cells or muscular cells
- ✓ *In situ* hybridization less sensitive than Real Time PCR ?

⇒ Better characterize cell types infected with the virus (TEM)

⇒ Better evaluate the impact of OsHV-1 on tissular architecture



Better understand host and virus responses during an infection

Better understand symptom/physiological dysfunction associated with the virus



Thanks for your attention

50  $\mu$ m

# Evolution of *Vibrios*, *Splendidus* group and OsHV-1 DNA in the 208 individuals

