Herpes virus OsHV-1

Clinical pathology

OsHV-1 (Oyster Herpesvirus type 1) infection causes mortality in the larvae and juveniles of several bivalve species including the Pacific oyster *Crassostreae gigas*, *Ostrea edulis*, *Ruditapes decussatus*, *R. philippinarum* and *Pecten maximus*. The virus can be found in adult bivalves (probably under a latent form) but without any mortality. Infected larvae show a reduction in feeding and swimming activities and mortality can reach 100% in a few days. Affected spat show sudden and high mortalities mainly during summer time. The virus is associated with abnormal nuclei through connective tissues, especially in mantle, labial palps, gills, and digestive gland. Since 2008, a variant (OsHV-1 µvar) has been identified and associated with high levels (up to 100%) of mortality in *Crassostrea gigas* spats reared in **France**(Segarra et al., 2010*)*, **Ireland** and **England***.* Mortality usually starts when water temperature reaches 16°C.

Recently OsHV-1 µvar was found associated with high mortalities of spat in **New Zealand** (November 2010) and high mortalities of spat and to a lesser extent of adult oysters *C. gigas* in **Australia** (December 2010). Note that Herpesvirus OsHV-1 very close to the OsHV-1 µvar from France were reported from **China** (2002) and **Japan** (2010). No mortalities were notified by these two countries. **Spain** and **Italy** reported the presence of OsHV-1 µvar without oyster mortality. In 2011 **The Netherlands** reported OsHV-1 µvar associated with mortalities in Pacific oysters*.*

An ostreid herpes virus (namely OsHV) has also been associated for several years with mortalities of juvenile Pacific oysters in Tomales Bay, California, **USA** (Friedmann et al., 2005).

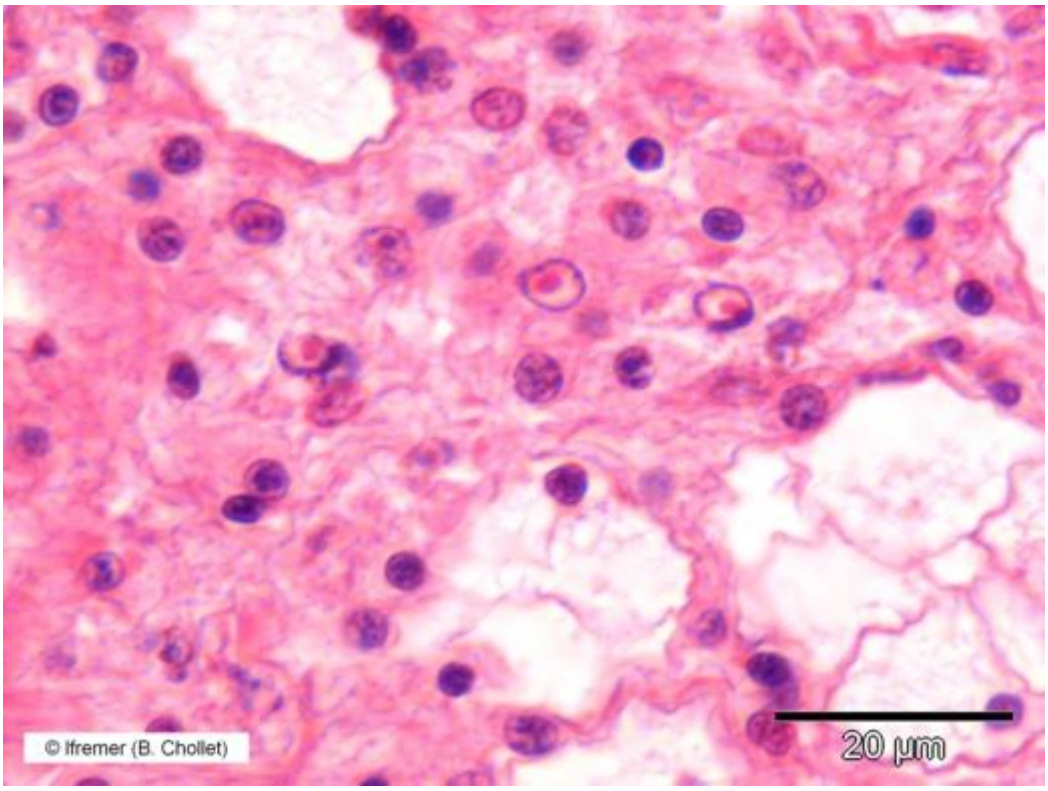
Agent description

OsHV-1 is a herpes-type virus or herpes-like virus. OsHV-1 from *Crassostrea gigas* reared in France has been described. However, the apparent lack of host specificity and loss of several gene functions in OsHV1 prompts speculation that this virus may have resulted from interspecies transmission in the context of introduction and intensive culture of non-native bivalve species (Arzul et al. 2001a, 2001b; ICES 2004). It is not known if the herpes-like viruses reported from various species of oysters and other bivalves are the same or different species of virus.

Screening techniques for the pathogen

Histology

Histology allows observing abnormalities however not specific to herpesviral infection. Cellular abnormalities are not associated with massive inflammatory reaction. Lesions are mainly observed in connective tissues in which fibroblastic-like cells exhibit enlarged nuclei with marginated chromatin (picture 1) and highly condensed nuclei in cells interpreted as hemocytes in spat.



Picture 1: Connective tissue from a flat Oyster *Ostrea edulis* showing cells with abnormal enlarged nuclei with marginated chromatin (H&E staining).

PCR

A **nested-PCR** using primers A3-A4 and A5-A6 and targeting (after the second amplification) 940 bp of a gene coding an unknown protein was first developed to detect the virus in Crassostrea gigas larvae and spat (Renault et al. 2000). Up to 500 fg of viral DNA can be detected in samples and these primers could not amplify other herpesviruses.

A **simple PCR** using primers C1-C6 (Renault and Arzul, 2001) has been then developed targeting 896 bp of a part of the viral genome located in an inverted repeat and coding fragments of unknown proteins. This protocol allows detecting up to 10 fg of viral DNA and these primers could not amplify other herpesviruses. This technique is often used for the detection of OsHV-1 especially in the context of abnormal mortalities. Larvae and spat are analysed by pool. This technique requires one day (from sample receipt to final results). Testing one pool of 5 juveniles costs about 6 € (including personal cost).

A **competitive PCR** method was also developed using previously designed primer pairs, C2-C6, amplifying a 710 fragment of the viral genome located in an inverted repeat and coding fragments of unknown proteins (Renault and Arzul 2001 and Renault et al. 2004). This technique is based on the use of oyster herpesvirus specific primers and an internal standard competitor that differs from the target DNA by a deletion of 76 bp. The assay allows detecting up 1 fg of viral DNA in 0.5 mg of oyster tissues. Moreover, this technique allows checking the presence of PCR inhibitors as well as performing a semi quantification of viral DNA.

Confirmatory techniques for diagnosis

PCR

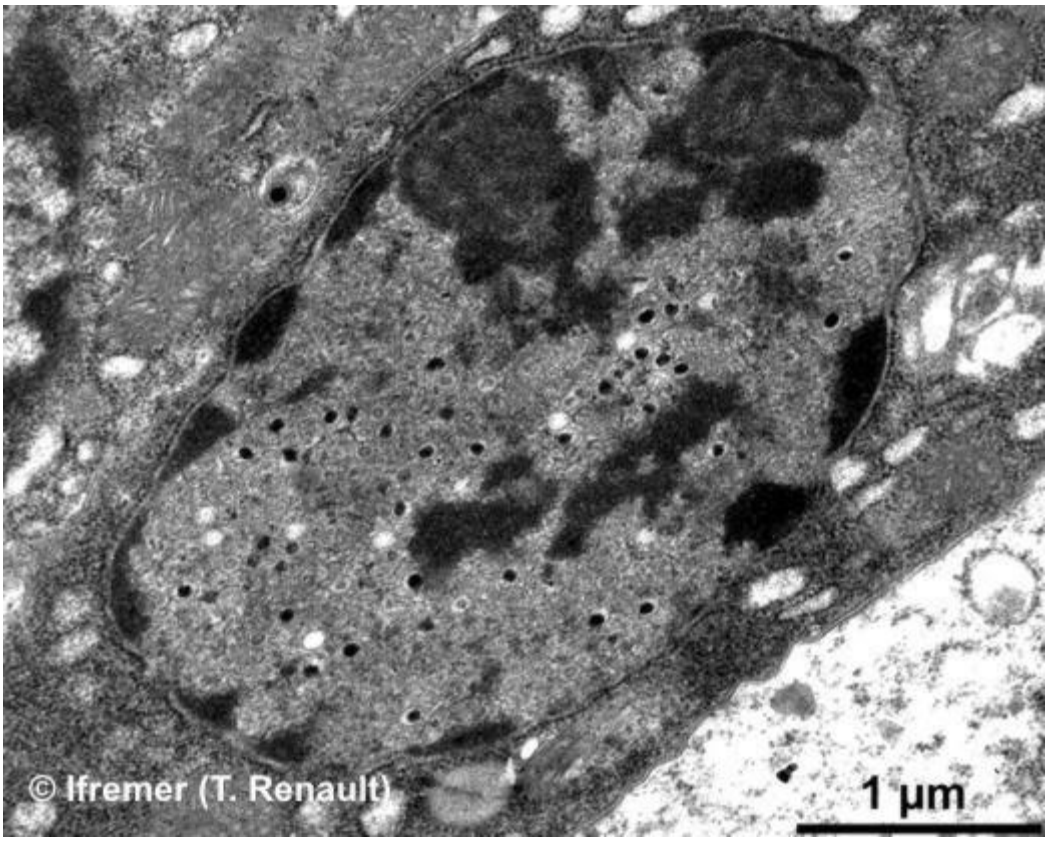
The different PCR protocols previously described in the section "screening techniques" can also be used as confirmatory techniques when suspicious lesions are observed by histology.

ISH

An *in situ* hybridization protocol has also been developed using dig-labelled A5/A6 and C1/C6 PCR products as probes (Lipart and Renault, 2002). Both probes were able to detect 50 pg of PCR amplified viral DNA by Southern Blot. No non-specific binding was observed when tests were performed on Human herpesvirus DNA. *In situ* hybridization is very convenient especially when infection level is low, like in adults. The test is performed on paraffin embedded tissues and requires 2 days before obtaining final results. The cost is estimated at 21 € for one individual (including personal cost).

TEM

Transmission electron microscopy is time consuming and cannot be applied in routine but is recommended when herpesvirus is suspected in a new host species. Viral particles are typical of members of the family Herpesviridae. Capsids and nucleocapsids can be observed in nuclei of infected cells while enveloped virions are present in the cytoplasm (picture 2).



Picture 2: TEM micrograph showing numerous herpes virus OsHV-1 in the nucleus of a heart tissue cell from *crassostrea gigas.* Note the enveloped virion outside the nucleus (upper left side of the picture).

Sequencing

Sequencing is recommended as one of the final steps for confirmatory diagnostic. The genome of OsHV-1 has been entirely sequenced and is available in Genbank (NC_005881 and AY509253). Obtained sequences should be compared with available ones in Genbank.

Comments and recommendations on available techniques

Protocols for PCR and *in situ* hybridization are available in pre cited articles. However both techniques need to be validated and more specifically specificity and sensitivity values are lacking.

What should we do for diagnosis at suspicion?

In case of suspicion in **larvae**: all dead and moribund larvae should be collected for DNA extraction and PCR according to Renault et al. 2000.

In case of suspicion in **juveniles**: Tests should preferably be performed on moribund individuals. 30 individuals should be analysed in pools of five animals. DNA extraction and PCR are performed according to Renault et al. 2000.

In case of suspicion in **adults**: OsHV-1 was never associated with mortality of adults. However, adults might be asymptomatic carriers. *In situ* hybridization can be used to test the presence of OsHV-1 in connective tissues of adults.

EU-legislation related to techniques

Not listed by the EU legislation.

OIE recommendations related to techniques

Not listed by the OIE Manual of Diagnostic Tests for Aquatic Animals (2009 version) nor by the Aquatic Animal Health Code (2009 version).

Assessment

The tests are discussed during the annual CRL/NRL meetings. Use the methods according to the table below for screening, and confirmation respectively.

Pathogen	Screening techniques (well established)	Confirmatory techniques (well established)	Evaluation
OsHV-1	Histology, PCR	PCR, DNA sequencing, ISH, TEM	No special further test needed

References

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FUENTE: **http://www.eurl-mollusc.eu/Main-activities/Tutorials/Herpes-virus-OsHV-1**