Ostreid herpesvirus: A pathogen of oysters

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Abstract
Ostreid Herpesvirus-1 (OsHV-1), OsHV-1 μvar and other variants are herpesviruses causing heavy mortalities, with losses ranging from 50 to 100%, on Pacific oyster (Crassostrea gigas), an important species worldwide cultured. Ostreid Herpesvirus-1 is one of the top 50 largest viral genome; OsHV-1 μvar is a variant of the virus OsHV-1 showing a systematic deletion of 12/13 base pairs sequence between ORF4 and ORF5. For the diagnosis of herpesvirus infection, traditional histopathological methods and biomolecular techniques have been developed, but, to confirm the disease, both methods should be used. This mini review reports some studies on the virus.

Introduction
Pacific oyster (Crassostrea gigas) is an important species worldwide cultured showing great production figures in many countries such as China, Japan, South Korea, U.S.A., Mexico, New Zealand, Australia, France, UK, Ireland, Spain, Italy, etc. However, oyster culture is periodically struck by heavy mortalities ranging from 50 to 100%. Oyster culture industry is based on both supply of the spat from the wild and from hatchery, where most of the larvae are produced using a “mass spawning” technique. Problems with low salinity, disease, or other water quality associated problems may delay or inhibit spawning. There are several different methods used for oyster growing. The methods range from culture on the bottom, to suspension in “poches”, baskets, etc., by rope and/or long-lines (Figure 1). Pacific cupped oysters will take from 18-30 months depending mainly on water temperature to reach a market size of 70–100 g live weight, > 75 mm shell length.

Losses of Pacific Oyster (C. gigas) have occurred globally for over 5 decades with heavy impact on oyster aquaculture. These losses generally denominated as Summer Mortality are typically prolonged and affect older reproductively mature animals during summer months [1]. In Europe, C. gigas was introduced to replace Crassostrea angulata, because significant summer mortalities have occurred since the late eighties [2-4]. The Pacific oyster may also be infected by different viruses; an irido-like virus has been reported infecting French Pacific oysters [5]. Although the Portuguese oyster, C. angulata, was highly susceptible to this virus, C. gigas appeared resistant to this virus disease. In late 70ies and early 80ies, high mortalities (more that 50%) of Pacific oyster larvae have been reported in commercial hatcheries from Washington State in USA [6]. The cause of C. gigas summer mortalities remained unclear, but studies suggested a complex etiology in which environmental and physiological conditions of the host (age/weight and sexual maturation) were involved [7,8].

Since 1992 sporadic high mortalities of larval C. gigas have been regularly observed in some commercial French hatcheries, occurring every year during summer in association with a herpes-like virus (Ostreid Herpesvirus 1: OsHV-1) [9,10]. In 2008 summer mortalities were reported at a much larger scale. The mortality events affected juvenile oysters and up to 100% mortalities were observed in a few weeks period between June and July. A new strain of OsHV-1 denominated μvar was consistently observed. Extensive mortalities were again observed in 2009 and from 2010 in all French coast and also in Ireland, Jersey and Italy.

Ostreid Herpesvirus
The Virus Study Group of the International Committee on Taxonomy of Viruses (ICTV) has recently established a new order, the Herpesvirales, which includes herpesviruses of mammals, birds, reptiles, amphibians, fish and bivalves. A characteristic of Herpesvirales is the latent or inapparent infection that could be present in host cells, but without replication. Genetically, Herpesviridae (mammals), Alloherpesviridae (amphibians) and Malacoherpesviridae (bivalves) are very few correlated. There is an unique protein, a terminase sub-unit responsible for DNA packaging, that could be considered Herpesvirus-specific. Based on terminase gene’s taxonomy-tree, the virus of oyster is a clade a part from the other Herpesvirus. In Figure 2 herpesviruses dataset and phylogenetic tree from Waltzek, et al. are shown [11].

Figure 1. Oyster “poches” culture in S. Teodoro Lagoon (Italy).
Malacoherpesviridae contains Ostreid Herpesvirus-1 (OsHV-1) as its sole member, in the genus Ostreavirus [12]. Ostreid Herpesvirus-1 is a virus which core contains a linear double-stranded DNA with an icosahedral capsid [13]. The sequence has been deposited in 2005 by Davison, et al. [14] and it is at 27th ranking of the top 50 largest viral genome sequence list. The sequence of the OsHV-1 is deposited in GenBank (GenBank AY509253), length estimated of 207 kbp: Davison et al. showed that the genome is assembled by two regions UL (167,843 bp) and US (3370 bp), each flanked by inverted repeats TRL/IRL (7584 bp) and TRS/IRS (9774 bp), and separated by a X region of 1510 bp [14].

OsHV-1 µvar

In the past decades, only Ostreid Herpesvirus 1 (reference type) and Ostreid Herpesvirus 1 µvar were known; then other C region variants have been detected. In 2008 an emerging variant, OsHV-1 µvar was isolated [15]. OsHV-1 µvar, shows a deletion in position 178204-178404 of about 200 bp which probably compromises the protein codified by ORF5, and there is an insertion of 27 bp not present in any published sequence (CCCCACTGTGATATCATCGAAATGAAT). In particular, OsHV-1 µvar is a variant of the virus OsHV-1 of 529 bp fragment length, which is defined on the basis of partial sequence data exhibiting a systematic deletion of 12/13 base pairs in TAC sequence between ORF4 and ORF5 in the position 178547 at 178572 of the genome (GenBank # HQ842610) in comparison with OsHV-1. Moreover, in some cases a deletion with only 10 bp in the same position was also observed. Furthermore, recent genomic regions analysis revealed the presence of at least nine different genotypes, including two variants close to the OsHV-1 (reference type) [16,17]. In particular, phylogenetic analysis results highlight two distinct clusters with a different geographical, European and East Asian, genotypes distribution.

Diagnosis

Nowadays, since there are not cell cultures from oyster and/or alternative cell culture, the viral isolation is not possible and there are no sensitive serological detection methods, but traditional histopathological methods and biomolecular techniques are used for the diagnosis of herpesvirus infection. However, to confirm the disease, both methods should be used.

Regarding the histopathological examination, what can be observed are the typical signs of a viral infection: connective tissue cells with pycnosis, nuclear changes (moon shaped), including nuclei hypertrophy with marginated chromatin, and large intranuclear acidophilic inclusion bodies (Cowdry type A inclusions) [10,18-21]. Moreover, the observation by electron microscopy of capsids or nucleocapsids in the nucleus and virus particles in the cytoplasm or in cytoplasmic vesicles could only confirm the presence of a herpesvirus-like and/or a viral disease [10,18-20,22-24].

On the other hand, both in situ hybridization (ISH) [25,26] and PCR could be positive for the DNA of OsHV-1 and variants detection but not enough for the disease confirmation. To date, there are numerous different PCR methods available for the detection of OsHV-1, but specific and characteristic are the primers C2 and C6, which bracket a region of 709 bp (178181-178889) [27-29] (Figure 3 and 4). Some methods of PCR...
between 1995 and 2007, did not detect the same virus [31,32]. Furthermore, OsHV-1 μvar was recently detected in Wadden sea [33] and in Adriatic sea [17].

Virus infectivity

Studies in vitro on virus stability and infectivity are very few. Ten pg µl-1 of extracted viral DNA were experimentally detected for 1, 9 and 16 day at 20, 11 and 4°C respectively and, in a second experiment, 100 pg µl-1 were detected after 51 days at each of the same temperatures [34]. However, the relationship between DNA detection in the PCR and infectivity is unknown. As a general rule of many aquatic animals viruses, considering also what observed in the field, the survival of OsHV is longer at lower temperatures.

OsHV virus, as other herpesviruses, could be present in host cells without replication, at latent or inapparent infection status and could be reactivated and recommences replication under a sum of environmental (such as water temperature, salinity, etc.) and host stressing conditions (i.e. age and sexual maturation of the host), etc. OsHV-1 was also detected in apparently healthy farmed oysters [27,29] and, again, in healthy farmed C. gigas from the Gulf of California [35]; while, regarding the oysters in the wild, the presence of OsHV-1 was recently confirmed in European natural C. gigas [17].

Water is probably the most important natural route of transmission of OsHV-1, since its DNA has been detected in the water around diseased Pacific oysters [36,37]. The experimental infection of Portuguese oyster, C. angulata, and Suminooe oyster, C. rivularis, has been performed by waterborne with extracted OsHV-1 or by cohabitation with infected C. gigas, respectively [38]. Furthermore, there are observations from both aquaculturists and researchers that the infection rate of young oysters results in higher mortality and morbidity than in older oysters, but infected adults may be the source of infection for larvae or spat, particularly if the oysters are in a stressing environment, e.g. high temperature [22,32,34]. Moreover, it is not confirmed if there is a vertical transmission other than horizontal one since the results were inconclusive [40].

Regarding the infectivity of OsHV(s) on marine molluscs, other than C. gigas, there are few data. The first record of a herpes-like virus in a bivalve mollusc in the USA was that described by Farley, et al. [41] in C. virginica. However, herpes-like virus have been reported in some molluscs such as C. ariakensis/C. rivularis, C. hongkongensis, C. sikamea [29], C. gigantea [42], Ostrea edulis [9], Pecten maximus [43], Ruditapes decussatus [38,44] R. Philippinarum [38,43,44], Saccostrea glomerata [45] and Tiosstreia chilensis [49]. In many cases, the identification of the virus was based on electronic microscopy and histopathological examinations of infected tissues consistent with the characteristics of that of herpesviruses, while before 2005 biomolecular examination was not always performed. Recently, Lopez-Sanmartin et al. (2016) demonstrated that O. edulis could be infected by OsHV-1 μvar by intramuscular injection. 

In conclusion, Pacific oyster (C. gigas) infection by OsHV-1, OsHV-1 μvar and other variants is cause of mortalities damaging aquaculture productions. There are various tools for diagnosis ranging from histopathological methods to biomolecular techniques such as in situ hybridization (ISH) and PCR and PCR Real-Time. However, as stated before, to confirm the disease, both methods should be used. Despite of diagnosis progresses, the problem on the field is currently unsolved: one measure now applied in the hatcheries is to produce virus free spat using parents checked to be virus free and kept in controlled water.
However, when the oysters are put in the sea, the disease can appear again, probably because the virus can be in the environment. Another way to fight the disease is the production of polyploid oysters: since they are triploid, because they have three sets of chromosomes, it is possible that they are less affected by the virus. However, when the oysters are put in the sea, the disease can appear again, probably because the virus can be in the environment. Another way to fight the disease is the production of polyploid oysters: since they are triploid, because they have three sets of chromosomes, it is possible that they are less affected by the virus.

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