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Ostreid herpesvirus: A pathogen of oysters

Daniela Mandas[1] and Fulvio Salati[1]*

[1]Fish Diseases and Aquaculture Center, IZS of Sardinia, State Veterinary Institute Via Parigi s.n., 09170 Oristano, Italy

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].

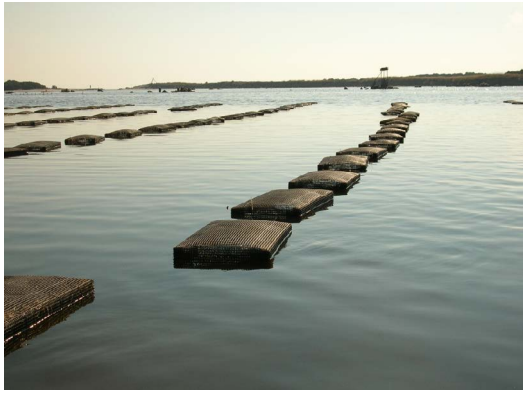

Figure 1. Oyster "poches" culture in S. Teodoro Lagoon (Italy).

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 carachteristic 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 phylogenic tree from Waltzek, *et al.* are shown [11].

Correspondence to: Salati F, Fish Diseases and Aquaculture Center, IZS of Sardinia, State Veterinary Institute Via Parigi s.n., 09170 Oristano, Italy, Tel: 328-6256492; E-mail: fulviosal@hotmail.com

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Species name (Virus abbreviation)	RefSeq
Alcelaphine herpesvirus 1 (AlHV1)	NC_002531
Ateline herpesvirus 3 (AtHV3)	NC_001987
Bovine herpesvirus 1 (BoHV1)	NC_001847
Bovine herpesvirus 4 (BoHV4)	NC_002665
Bovine herpesvirus 5 (BoHV5)	NC_005261
Ceropithecine herpesvirus 2 (CeHV2)	NC_006560
Ceropithecine herpesvirus 9 (CeHV9)	NC_002686
Equid herpesvirus 1 (EHV1)	NC_001491
Equid herpesvirus 4 (EHV4)	NC_001844
Human herpesvirus 1 (HHV1)	NC_001806
Human herpesvirus 2 (HHV2)	NC_001798
Human herpesvirus 3 (HHV3)	NC_001348
Human herpesvirus 4 (HHV4)	NC_001345
Human herpesvirus 5 (HHV5)	NC_006273
Human herpesvirus 6 (HHV6)	NC_001664
Human herpesvirus 7 (HHV7)	NC_001716
Human herpesvirus 8 (HHV8)	NC_003409
Gallid herpesvirus 1 (GaHV1)	NC_006623
Gallid herpesvirus 2 (GaHV2)	NC_002229
Gallid herpesvirus 3 (GaHV3)	NC_002577
Macacine herpesvirus 1 (McHV1)	NC_004812
Macacine herpesvirus 4 (McHV4)	NC_006146
Macacine herpesvirus 8 (McHV8)	NC_006150
Meleagrid herpesvirus 1 (MeHV1)	NC_002641
Murid herpesvirus 2 (MuHV2)	NC_002512
Murid herpesvirus 4 (MuHV4)	NC_001826
Ovine herpesvirus 2 (OvHV2)	NC_007646
Ostreid herpesvirus 1 (OsHV1)	NC_005881
Panine herpesvirus 2 (PaHV2)	NC_003521
Psittacid herpesvirus 1 (PsHV1)	NC_005264
Saimiriine herpesvirus 2 (SaHV2)	NC_001350
Suid herpesvirus 1 (SuHV1)	NC_006151
Tupaiid herpesvirus 1 (TuHV1)	NC_002794
T4 bacteriophage (T4)	NC_000866
RB 69 bacteriophage (RB69)	NC_004928

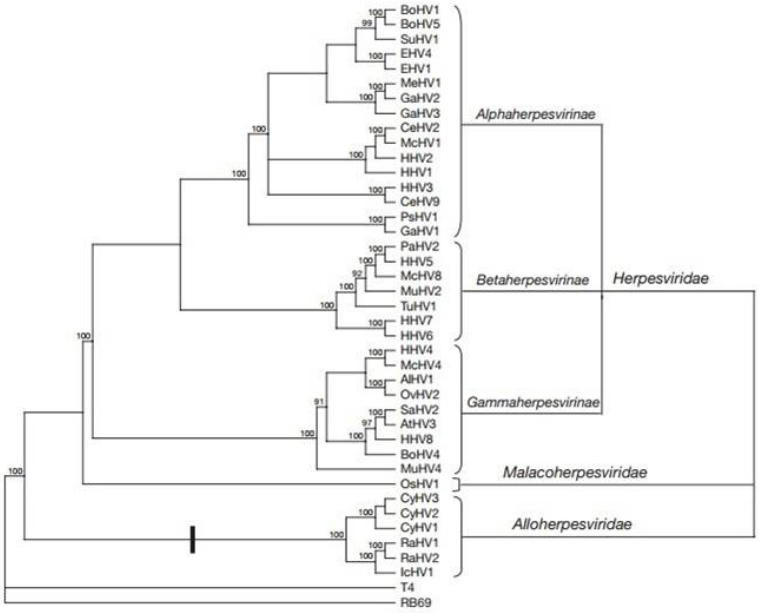

Figure 2. Herpesviruses dataset and phylogenic tree [11].

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 capside [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), lenght 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 1var 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 (CCCACTGTGATATCATCGCAAATGAAT). 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 posion 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 hands, 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

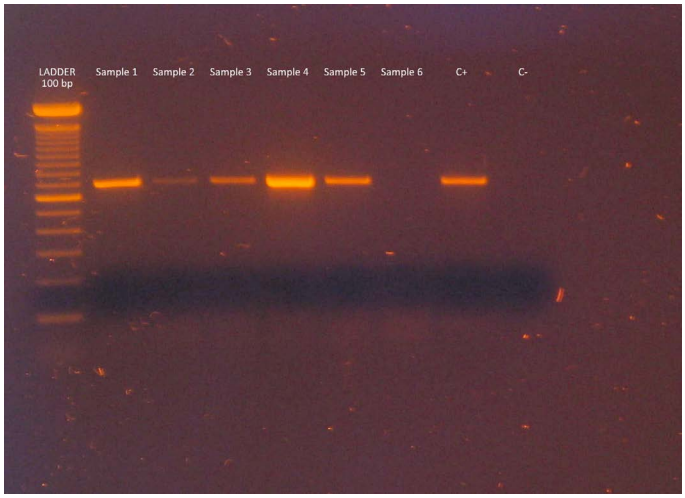

Figure 3. Example of OsHV-1 gel from diseased C. gigas. The gel shows the reference virus and virus-negative oyster tissue. Es. Samples line 1 to 5: positive; Sample line 6: negative; C+ = reference OsHV-1; C- =negative.

Figure 4. The lover of oysters.

utilize a PCR where the DNA sequence is amplified by one primer pair, other methods use nested PCR with two amplification using two primer pairs: primer sets C2-C4, C2-C6 [28] and C2-C6 and CF-CR. The late should be used to clearly identify the OsHV-1 µvar strain from the reference strain by comparison with positive controls [30]. Furthermore, sometimes it is difficult to distinguish, on the electrophoresis gel, between the reference and the µvar; particularly, in case of double infection, the bands may overlap and form a broad band. Moreover, CF-CR primers could produce a ghost band from host DNA which has been identified by sequencing. The PCR may not be sensitive enough to detect virus at very low levels found in latent infections and, as a consequence, the Real-Time method using the CF-CR results to be more accurate to distinguish the strain. Therefore, some laboratories prefer to sequence the PCR product.

From 2008-2009, OsHV-1 µvar was detected in association with high mortality events in many European countries, while analysis conducted in samples associated to mortality events prior to 2008, 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 summ 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 trasmission 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 Suminoe 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 *Tiostrea 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 spats 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 better survival was recorded using triploid oyster [47], because they commonly have lower gonadal activity, are less fertile than diploids [48] and spare energy to more effectively fight the viral disease. Recently together to polyploid production, studies on genetic selection with the purpose of obtaining a more genetically resistent oysters strain/family have started [49,50-52]. Moreover, studies on oyster immune response to Ostreid Herpesviruses and newly appeared pathogenic bacteria, such as *Vibrio aestuarianus*, [53,54] are now developing [55-67].

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