

Shellfish viruses

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Summary

Shellfish cultivation may be endangered by the occurrence of epizootic viral diseases. Mortalities in a number of commercially important mollusc species have been associated with the detection of viruses belonging to several families. The first description of a virus was in adult eastern oysters, *Crassostrea virginica*, with the detection of virus particles resembling members of the family *Herpesviridae*. Subsequently, mass mortalities in French stocks of adult Portuguese oysters, *C. angulata*, were associated with irido-like virus infection. Other viruses observed in molluscs are described as members of the families *Iridoviridae*, *Papovaviridae* and *Reoviridae*. Little information is available on viral infections that affect molluscs due to primarily to the inadequacy of diagnostic methods that are employed when mass mortality events occur. Most laboratories involved in mollusc pathology still employ histopathology for the analysis of samples. *Ostreid herpesvirus 1* (OsHV-1) is the only well characterised shellfish pathogen with complete genome sequence now available.

Key words: abalones, birnavirus, bivalves, clams, herpesvirus, diseases, irido-like virus, molluscs, oysters, oyster herpesvirus 1, picornavirus, scallops, virus

Glossary

Shellfish – aquatic invertebrates belonging to the crustacean or mollusc families and used as food.

Mollusc – invertebrates having a soft unsegmented body usually enclosed in a shell.

Bivalve – marine or freshwater molluscs having a soft body with plate-like gills enclosed within two shells hinged together.

Aquaculture – rearing aquatic animals or cultivating aquatic plants for food

Gills – respiratory organ of aquatic animals that breathe oxygen dissolved in water.

Mantle – a protective layer of epidermis in molluscs that secrete a substance forming the shell.

Hemocyte – any blood or formal element especially in invertebrates

Hatchery – a place where eggs are hatched under artificial conditions.

Larva – the immature free-living form of most invertebrates with at hatching from the egg is fundamentally unlike its parent and must metamorphose.

Velum – membrane of mollusc larvae that allows swimming activity.

Nursery – A place for the cultivation of juveniles under controlled conditions.

Introduction

A natural abundance of shellfish was common in many areas of the world until the early twentieth century. However, industrial and urban development and population growth in coastal areas, coupled with extreme harvest pressure, appear to have contributed to a steady decline in natural shellfish populations. This decline in wild harvests, together with a greater demand for seafood from an increasing world population, have driven the development of technology for the intensive management and cultivation of shellfish. As a result, global shellfish production, the greatest proportion of which is bivalves, was estimated to be 10,732,000 metric tons in 2000. However, as husbandry practices have developed, the significant impact of infectious diseases on productivity and product quality has been increasingly recognized. Numerous examples worldwide have demonstrated that entire shellfish industries in coastal areas are susceptible to disease and that the production of healthy shellfish is a key to the economic viability of mollusc farming.

The study of shellfish diseases is a relatively young science and the discovery of viruses in marine molluscs is a fairly recent event. Viral diseases have seriously affected the aquaculture industry during last decades. Viral pathogens are often highly infectious and easily transmissible, and are commonly associated with mass mortalities. Viruses interpreted as members of the families *Iridoviridae*, *Herpesviridae*, *Papovaviridae*, *Reoviridae*, *Birnaviridae* and *Picornaviridae* have been reported as associated with disease outbreaks and causing mortality in various molluscs. However, there is currently a lack of information concerning the occurrence of mollusc viruses world-wide and the basic method for identification and examination of suspect samples is still predominantly histopathology. This technique enables the identification of cellular changes associated with infection but does not provide conclusive identification of mollusc viruses unless completed by other methods such as transmission electron microscopy. Moreover, as there is a lack of marine mollusc cell lines and since invertebrates lack antibody-producing cells, the direct detection of viral agents remains the only possible approach to diagnosis.

As filter feeders, bivalves may also bioaccumulate viruses from humans and other vertebrates, acting as a transient reservoir. The consumption of raw or under-cooked shellfish can result in human disease and contamination of shellfish cultivated in coastal marine waters by microorganisms that are pathogenic to humans is a worldwide public health concern. The association of shellfish-transmitted infectious diseases with sewage pollution has been well documented since the late 19th and early 20th centuries. Human enteric viruses including rotaviruses, enteroviruses and hepatitis A virus are the most common etiological agents transmitted by shellfish. These enteric viruses are associated with several human diseases ranging from ocular and respiratory infections to gastroenteritis, hepatitis, myocarditis, and aseptic meningitis. Many of these viruses are transmitted by the fecal-oral route and are widely prevalent in locations with poor sanitation. There is considerable literature on the human health implications. However, this is not the subject of the present chapter.

Irido-like viruses

Hosts and locations

Irido-like virus infections have been reported in oysters in France and in the USA. Two distinctive conditions have been associated with mass mortalities in adult Portuguese oyster, *Crasostrea angulata*, along French coasts: gill necrosis virus disease and hemocytic infection virus disease. A viral infection similar to the latter was reported in the Pacific oyster *C. gigas* during summer mortalities in the Bay of Arcachon (Atlantic coast, France) long after the disappearance of the Portuguese oyster. A third type of irido-like virus, the oyster velar virus (OVV), has been reported from hatchery-reared larval Pacific oysters on the west coast of North America (Washington State, USA).

Disease manifestations and epizootiology

Gill necrosis virus disease is regarded as the primary cause of disease outbreaks and mortalities occurring in the late 1960s among Portuguese oysters on the Atlantic coast of France. The disease appears to have affected up to 70% of oyster populations with maximum losses reported in 1967. Losses subsequently declined and survivors recovered from the disease. The first gross signs were small perforations in the center of yellowish discolored zones of tissue on gills and labial palps. Further development and extension of the lesions resulted in larger and deeper ulcerations. In advanced stages, total destruction of affected gill filaments was observed. Yellow or green pustules also developed on the adductor muscle and mantle.

In 1970, high mortality rates were again reported in *C. angulata* oysters in France. Mortality was first observed in the estuary of Marennes Oleron (Altantic coast) and in Brittany. The high mortality rates occurring during this epizootic led to almost total extinction of French *C. angulata* by 1973. The disease affected adult oysters. No distinctive clinical signs were noted (eg. no gill lesions). Histological observations included an acute cellular infiltration consisting of atypical virus-infected hemocytes. Pacific oysters seemed to be resistant to the vius and subsequently replaced *C. angulata* in France. However, a morphologically similar virus was reported from Pacific oysters during an outbreak of summer mortality in the Bay of Arcachon (Atlantic coast, France) in 1977. Although affected Pacific oysters exhibited virtually no gross signs, the presence of atypical cells interpreted as infected hemocytes and degeneration of connective tissues were reported in infected animals.

Oyster velar virus disease (OVVD) of the Pacific oyster occurred in the USA from mid-March through mid-June each year from 1976 to 1984, suggesting that the expression of the disease may be related to particular environmental. When cultured at 25-30°C, mortalities in oysrter larvae greater than 170 µm in shell length typically begins at about 10 days of age. The infection results in the sloughing of ciliated velar epithelial cells and detachment of infected cells from the velum. Other cells lose cilia and infected larvae become unable to move normally.

Descriptive histopathology

Histologically, gill necrosis virus disease is characterized by tissue necrosis with massive hemocytic infiltration aroud the lesions. The most distinctive lesion is the occurrence of giant polymorphic cells which may be up to 30µm in size and contain large fuchsinophilic granules in

the cytoplasm. In some giant cells, a voluminous basophilic inclusion (5-15 μm) occupies the greatest part of the cytoplasm in which finer basophilic granules (0.4-0.5 μm) are also present. Electron microscopy indicates that the inclusions are the viroplasm and the fine granules are large viral particles. The most characteristic histological lesion of hemocytic infection virus disease is an acute cellular infiltration with the presence of atypical hemocytes in the connective tissues. Basophilic intracytoplasmic inclusion bodies are found in atypical blood cells in which irido-like virus particles can be observed in ultrathin sections. OVVD disease manifests histologically by the presence of intracytoplasmic inclusion bodies, 1.2-4 μm in diameter, located most commonly in the ciliated velar epithelium. The presence of DNA in the inclusion bodies is suggested by a positive Feulgen and Rosenbeck reaction.

Viruses

Mature icosahedral virions (380 nm diameter) are scattered through the cytoplasm of infected cells <Figure 1A near here>. The outer shells of virions appear to consist of two trilaminar layers. The electron-opaque core (250 nm diameter) is limited by a three-layered fringe of definite width and surrounded by a layer of dense material <Figure 1B near here>. Morphogenesis takes place in the cytoplasm. Oyster irido-like viruses have not been isolated from infected tissue and have not been characterized biochemically. However, the presence of deoxyribonucleic acid (DNA) viruses was demonstrated by histochemical techniques including acridine orange staining and the Feulgen and Rosenbeck reaction. The characteristic morphology and cytoplasmic localisation of these large DNA viruses suggests that they may eventually be classified as members of the family *Iridoviridae*. However, no molecular characterization has yet been conducted and there remains a need for definitive demonstration of viral etiology for the reported diseases.

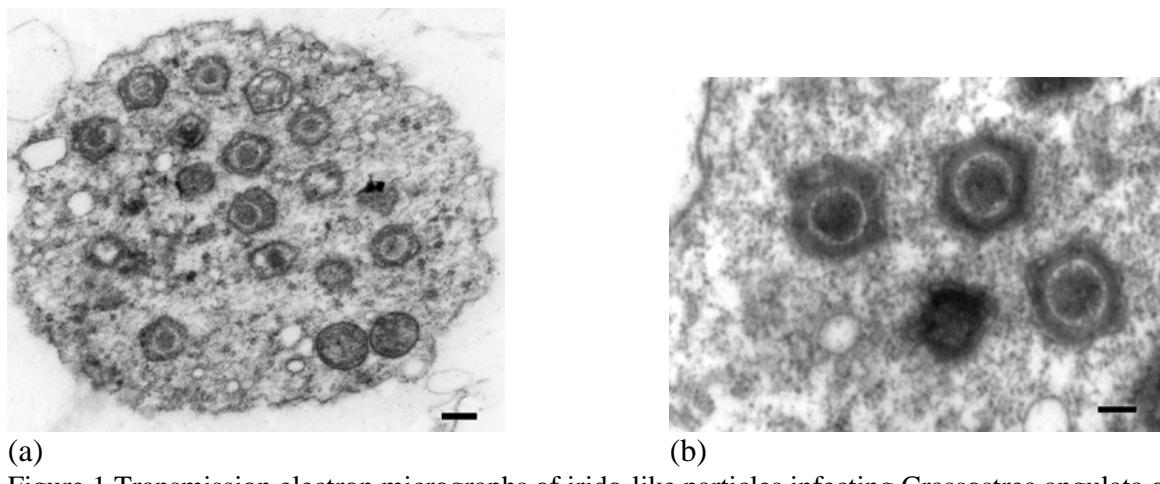


Figure 1 Transmission electron micrographs of irido-like particles infecting *Crassostrea angulata* oysters.
 (a) Intracytoplasmic irido-like virus particles in an infected *C. angulata* cell (gill necrosis virus disease).
 Scale = 200 nm. (b) Irido-like virus particles from *C. angulata*. Virions are icosahedral in shape with a central electron-dense core, surrounded by an electron-lucent zone followed by another dense layer. Two unit membranes separated by a clear zone enclose the particle. Scale 1/400 nm.

Herpesviruses

Hosts and locations

Herpes-like virus infections have been identified in various marine mollusc species throughout the world, including the USA, Mexico, France, Spain, the UK, New Zealand, Australia, and Taiwan. The first description of a virus morphologically similar to members of the family *Herpesviridae* in a bivalve mollusc was reported in 1972 in the eastern oyster, *Crassostrea virginica*. Since then, a wide host range has been reported for herpes and herpes-like viruses infecting bivalve species, including the Pacific oyster *C. gigas*, the European oyster *Ostrea edulis*, the Antipodean flat oyster *O. angasi*, the Chilean oyster *Tiostrea chilensis*, the Manila clam *Ruditapes philippinarum*, the carpet shell clam *R. decussatus*, the Portuguese oyster *C. angulata*, the Suminoe oyster *C. rivularis* and the French scallop *Pecten maximus*. It is noteworthy that recently a herpes-like virus has also been observed by transmission electron microscopy in the gastropod mollusc *Haliotis diversicolor supertexta* in Taiwan associated with high mortality rates.

Disease manifestations and epizootiology

Herpesvirus and herpes-like virus infections have been associated with high mortalities of hatchery-reared larvae and juveniles stages of several bivalve mollusc species . Observations by transmission electron microscopy indicate that larvae exhibit generalized infections, whereas focal infections usually occur in juveniles. Although viral infections have also been observed in adult bivalves, they are apparently less sensitive than younger stages. Infected larvae exhibit velar and mantle lesions. They swim weakly in circles and shortly before death settle at the bottom of the tanks. Infected juveniles exhibit sudden high mortalities in a short period of time (less than one week) often during the summer time. Histologically, lesions are confined to connective tissues. Fibroblastic-like cells exhibit abnormal cytoplasmic basophilia and enlarged nuclei with marginated chromatin. Other cell types including hemocytes and myocytes show extensive chromatin condensation. Peculiar patterns of chromatin with a ring-shape or crescent-shape are also observed suggesting that apoptosis may occur. Viral DNA and proteins have been detected in asymptomatic adult oysters. Like other herpesviruses, the *C. gigas* herpesvirus seems to be capable of long term persistence in the infected host. The pathogenicity of the virus for the larval stages of *C. gigas* has been demonstrated by experimental transmission to axenic larvae. Attempts to reproduce symptoms experimentally in juveniles and adult oysters have so far been inconclusive.

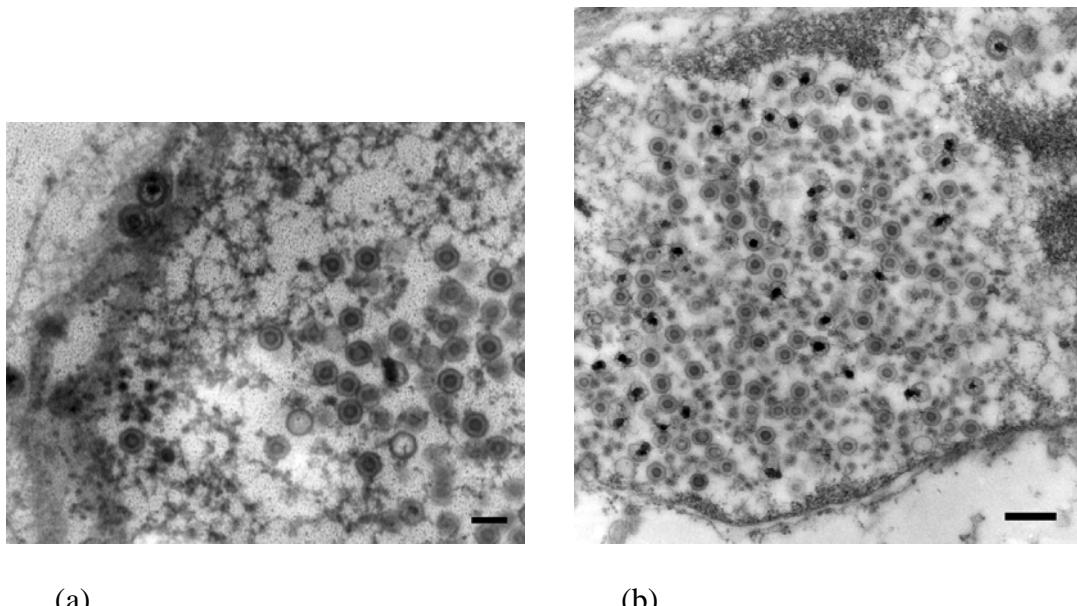
Virus ultrastructure

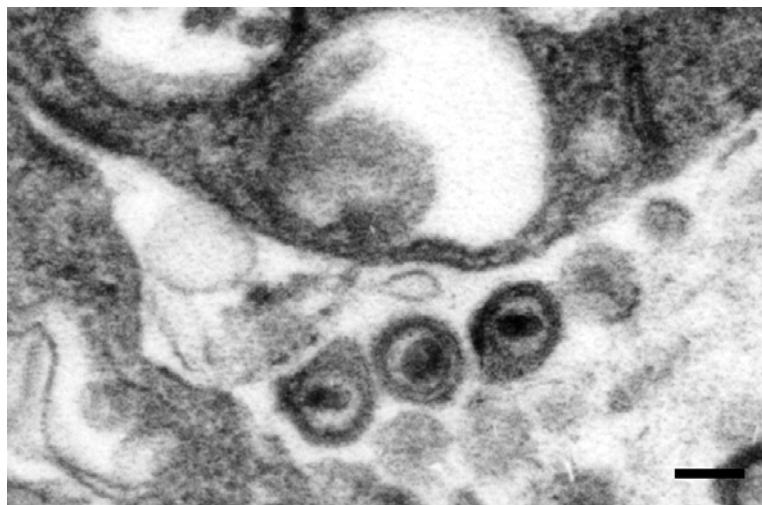
Based primarily on virion morphology and aspects of morphogenesis and genome organisation, *Ostreid herpesvirus 1* (OsHV-1) is currently classified as an unassigned species in the family *Herpesviridae*. Particles present in the nucleus are circular or polygonal in shape. Empty and are presumed to be capsids others containing an electron-dense toroidal or brick-shaped core are

interpreted as nucleocapsids <Figure 2A near here>. Capsids and nucleocapsids are scattered throughout the nucleus in infected cells <Figure 2B near here>. An electron-lucent gap of approximately 5 nm with fine fibrils is observed between core and capsid. Digital reconstruction of the OsHV-1 capsid based on cryo-electron microscopic images indicates an icosahedral structure with a triangulation number of T=16 which is an architecture unique to herpesviruses. Prominent external protrusions at the hexon sites, and a relatively flat and featureless appearance of the inner surface reported for OsHV-1 capsids are also characteristic features of herpesviruses. Extracellular particles are usually enveloped formed by a trilaminar unit-membrane and measure 100 to 180 nm in diameter <Figure 2C near here>. Tegument between the outer membrane and the capsid shell of enveloped particles is either absent or reduced <Figure 2C near here>.

Genome structure and organization

Virus particles have been purified from fresh infected *C. gigas* larvae and the entire OsHV-1 genome has been cloned and sequenced (GenBank accession number AY509253). The total genome size is 207439 bp. The overall genome organization is TR_L - U_L - IR_L - X - IR_S - U_S - TR_S <Figure 6 near here> in which TR_L and IR_L (7584 bp) are inverted repeats flanking a unique region (U_L, 167843 bp), TR_S and IR_S (9774 bp) are inverted repeats flanking a unique region (U_S, 3370 bp), and X (1510 bp) is located between IR_L and IR_S. A somewhat similar genome structure has been reported for certain vertebrate herpesviruses (e.g. herpes simplex virus and human cytomegalovirus). A small proportion of OsHV-1 genomes either lack the X sequence or contain an additional X sequence at the left terminus.





(c)

Figure 2 Transmission electron micrographs of ostreid herpesvirus 1 (OsHV-1) infecting Pacific oyster larvae. (a) Intranuclear spherical or polygonal virus particles; some particles appear empty and other contain an electron-dense core. Scale = 100 nm. (b) Nucleus of an infected interstitial cell containing empty capsids and nucleocapsids. Scale = 200 nm. (c) High magnification of extracellular enveloped particles. Scale = 100 nm.

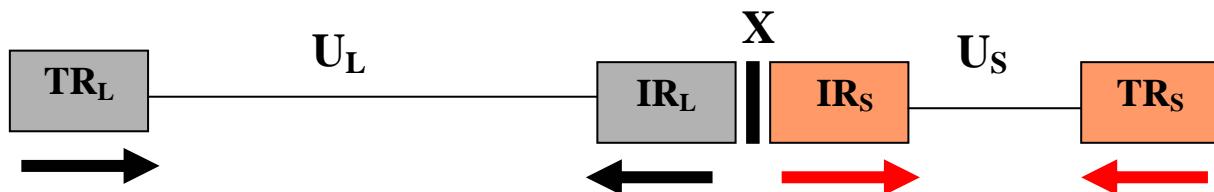


Figure 3. General genome organization of ostreid herpesvirus 1 (OsHV-1). TR_L and IR_L are inverted repeats flanking the unique region U_L . TR_S and IR_S are inverted repeats flanking the unique region U_S .

Since herpesvirus genomes are packaged into capsids from head-to-tail concatemers, this minor genome form may result from rare cleavage of concatemers at $X - TR_S$ rather than at $IR_L - IR_S$. Moreover, approximately 20-25% of genomes contain a 4.8 kbp region of U_L in inverse orientation. The two orientations of U_L and U_S are present in approximately equimolar amounts in viral DNA, giving rise to four genomic isomers. This is also a feature of the vertebrate herpesvirus genomes with similar structures and results from recombination between inverted repeats during DNA replication. The genome termini are not unique but a predominant form is apparent for each. The $IR_L - IR_S$ junction is also not unique, but the predominant form corresponds to a fusion of the two termini if each possesses two unpaired nucleotides at the 3' end. Unpaired nucleotides are characteristic of herpesvirus genome termini.

Detailed analysis of the OsHV-1 genome sequence indicates there are 124 unique open reading frames (ORFs). Owing to the presence of inverted repeats, 12 ORFs are duplicated resulting in a total of 136 genes in the viral genome. These numbers include several fragmented genes, each of

which is counted as a single ORF. It is not yet known if splicing contributes to further elaboration of gene expression. A total of 38 genes shares sequence similarities with other genes of the virus, defining 12 families of related genes. These include one gene family encoding proteins containing helicase motifs, one family encoding product related to inhibitors of apoptosis (IAPs), one family derived from a deoxyuridine triphosphatase gene, three families predicted to encode membrane-associated proteins, two families encoding RING-finger proteins, two families whose products are predicted to be secreted and two other families. Gene families are also present in all other sequenced herpesviruses. The observation that IAPs are also encoded by baculoviruses and entomopoxviruses (both of which have insect hosts) underscores the importance of the apoptotic responses of invertebrates against viral infections. Vertebrate herpesviruses and poxviruses do not encode IAPs, and subvert the battery of host defences by other pathways. Amino acid sequence comparisons have provided functional information on 25 genes that are not members of families. Seven genes encode enzymes. These include the catalytic subunit of DNA polymerase, two subunits of ribonucleotide reductase, a helicase, a putative primase and the ATPase subunit of terminase. Two additional genes encode RING-like proteins. One protein is related to a eukaryotic protein of unknown function which is brain-specific in vertebrates. The ORF30 protein is related in an N-terminal cysteine-rich domain to a protein of unknown function in mammalian herpesviruses. A total of 15 genes encode proteins which have predicted signal or transmembrane sequences and therefore may be associated with membranes, one specifying a putative ion channel.

Evolution and taxonomy

Even though OsHV-1 shares a similar capsid architecture, amino acid sequence comparisons have failed to identify a single protein which has homologues in proteins unique to herpesviruses, with the exception of ORF30 which contains a domain found to date only in a subset of mammalian herpesviruses. Several OsHV-1 proteins have homologues that are distributed widely in nature (e.g. DNA polymerase), but these are no more closely related to homologues in other herpesviruses than to homologues in other organisms. This finding is also characteristic of comparisons between herpesviruses which infect fish or amphibians and those that infect mammals or birds. The strongest genetic indication of a common origin resides with the ATPase subunit of the terminase, which is involved in packaging DNA into the capsid. Homologs of this gene are present in all herpesviruses and T4 and related bacteriophages. The T4 and OsHV-1 genes are unspliced, whereas those in herpesviruses of mammals and birds contain one intron and those in herpesviruses of fish and amphibians contain two introns. The available data support the view that herpesviruses of mammals and birds, herpesviruses of fish and amphibians and herpesviruses of invertebrates form three major lineages of the herpesviruses. OsHV-1 would have established a separate lineage about a billion years ago, and the fish viruses about 400 million years ago. OsHV-1 is currently the single representative of what may be a large number of invertebrate herpesviruses. Recent data show that OsHV-1 can infect several bivalve species. This contrasts with vertebrate herpesviruses, which are generally confined to a single species in nature. Consequently, the true host of OsHV-1 is unknown. The apparent loss of several gene functions in OsHV-1 prompts the speculation that this may have promoted interspecies transmission in the context of introduction of non-native bivalve species and use of modern aquaculture techniques.

Diagnosis and epidemiological surveys

Light microscopy remains the preferred method for diagnosis of herpes-like virus infections in suspect samples. However, this method is poorly suited to diagnosis of viral diseases and should be supported by other techniques such as transmission electron microscopy. Even so, microscopic techniques are time consuming and unsuitable for epidemiological surveys. The lack of bivalve cell lines does not allow in vitro culture and the observation of virus cytopathogenic effects. The purification of OsHV-1 from fresh infected larval *C. gigas* has served as a platform for generation of molecular biological reagents for diagnosis. Procedures to detect herpes-like viruses in oysters using polymerase chain reaction (PCR) and *in situ* hybridisation (ISH) have been developed and are suitable for epidemiological surveys of field samples, such as are currently being performed on oyster spat and larvae from commercial hatcheries and shellfish farms in France.

Picorna-like viruses and other small virus-like particles

Virus-like particles of 27 nm in diameter have been reported in *Mytilus edulis* mussels from Denmark. The virus-like particles were enclosed in vesicles and arranged singly or in paracrystalline arrays. Acute cellular infiltrations were associated with virion detection and were interpreted as granulocytomas. Electron -dense, unenveloped virus-like particles (25-45 nm) have also been detected in farmed *Perna canaliculus* and *M. galloprovincialis* mussels suffering mortalities in New Zealand. Extensive haemocytosis and necrosis of interstitial cells, basal cells and digestive tubule epithelial cells were observed. Small DNA-negative virus-like particles (22 - 30 nm) were also reported in digestive and secretory cells of scallops, *Pecten novaezelandiae*, and toheroa, *Paphies ventricosum*, from New Zealand. Mass mortalities in Japanese pearl oysters, *Pinctada fucata martensii*, which have occurred in Japan since 1994 have been associated with a non-enveloped virus (25-33 nm) called Akoya virus. The disease was characterised by necrosis and degeneration of muscle fibers. A morphologically similar virus was detected in the pearl oyster, *P. margaritifera*, from French Polynesia associated with granulomas and focal necrosis within the adductor muscle. These lesions were similar to those reported in the mussel *M. edulis* suffering granulocytomes. Paraspherical or polygonal shaped virus-like particles (40 nm) consisted of a membrane-like envelope coating a central 35 nm electron-dense core. Icosahedral-spherical (27 to 35 nm), non-enveloped virus particles have also been detected in the cytoplasm of connective tissue cells from cultured carpet-shell clams, *Ruditapes decussatus*, suffering mortalities in Galicia (Spain). More recently, non-enveloped, icosahedral (19-21 nm) virus-like particles have been associated with large foci of massive hemocytic infiltration in cockles, *Cerastoderma edule*, from the same area (Galicia, Spain). The detection of red deposits on histological sections after methyl green pyronin staining suggested this is an RNA virus. All these virus-like particles are similar to those described first in mussels from Denmark. Due to their size, morphology and the formation of paracrystalline arrays they have been supposed to belong to the family *Picornaviridae*. However, no molecular information is available on these viruses to date.

Papova-like viruses

Intranuclear, non-enveloped virus-like particles (50 to 55 nm) with an icosahedral symmetry were first reported from the gonadal epithelia of *C. virginica* in the USA in 1976 and in the Canada in 1994. The virions were interpreted as papova-like viruses. A papova-like virus has also been detected in gonadal tissues during a health survey of cultured Pacific oyster, *C. gigas*, from the southern coast of Korea,. More recently, histological examination of *C. gigas* oysters in France has revealed several cases of abnormally large basophilic cells in gonadal tissues. Electron microscopy examination has revealed non-enveloped icosahedral particles 50 nm in diameter.

Reo-like viruses and birnaviruses

A virus tentatively assigned the family *Reoviridae* has been isolated from juvenile eastern oysters, *C. virginica*, using a fish cell line. Virions have been described as slightly oval particles (79 nm in diameter) containing a distinct inner core and clear spike-like projections on the outer capsid. Birnaviruses have been isolated from different bivalve species in Europe and Taiwan. A virus tentatively named “Marine birnavirus” has also been isolated during a high mortality episode from oysters cultured in the Uma Sea (Japan). Marine birnaviruses (MABV) have been defined as a group within the genus *Aquabirnavirus*. Although the pathogenicity of certain MABV strains appears to be weak in shellfish, it has been observed in some mollusc species (the clam *Meretrix lusoria*, the Agemaki or jack knife clam *Sinovacula constricta*, and the Japanese pearl oyster, *P. fucata*) that stressors such as changes in temperature, spawning and exposure to heavy metals can result in mortality by increasing host susceptibility. MABV may thus be an opportunistic pathogen which persistently infects marine organisms and becomes pathogenic under stressful conditions. Birnavirus-like particles were also isolated from the thin telling, *Tellina tenuis* and the flat oyster, *Ostrea edulis* from the coast of Britain and east coast of Canada. MABV isolated from shellfish appear to be pathogenic to fish. Moreover, based on serological and genomic properties, strains isolated from shellfish and fish seem similar. This may indicate that the host range of MABV may be broad. Assays to reproduce experimentally the infection using the reovirus-like and birnavirus-like particles isolated on a fish cell line, have shown inconsistent results and a firm conclusion on the significance of these viruses for shellfish is still unknown.

Conclusion

In some cases, mollusc viruses have been detected only as inconsequential infections in animals that are suffering from another known disease or from an environmental stress such as pollution. However, several mass mortality outbreaks in molluscs have been attributed to viral infections. The almost total extermination of the Portuguese oyster, *C. angulata*, in French and European Atlantic waters in 1973 has been associated with irido-like virus infections. Viruses morphologically similar to members of the *Herpesviridae* have also been associated with high mortality rates in various marine mollusc species around the world.

The production of healthy shellfish from hatcheries and nurseries is a critical aspect of the conservation and management of natural populations and extensive farming areas. Selective

breeding of hatchery stock will also be important for aquaculture development. This may lead to a substantial international trade in bivalve gametes and larvae to allow distribution of genetically improved seed stock. However, significant production problems including the elimination of viral diseases must be solved before hatcheries can become a major supplier for the industry. High density production systems including commercial hatcheries and nurseries are an important source of viral diseases in aquaculture and the movement of stock must be considered as one of the major risks of disease spread. The risk of viral disease in invertebrate aquaculture species is accentuated by the lack of specific chemotherapies and vaccines. Improved knowledge and understanding of shellfish viruses is needed in order to develop new tools for disease control.

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