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French Scallops: A New Host for Ostreid Herpesvirus-1

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Abstract:

Sporadic high mortalities were reported among larval French scallops (*Pecten maximus*). Electron microscopy of moribund larvae revealed particles with the characteristics of a herpesvirus in association with cellular lesions. PCR and DNA sequencing showed that the virus is a variant of ostreid herpesvirus-1 that has already been described in clams and oysters. This is the first description of a herpesvirus infection of a scallop species. The virus was transmitted successfully from an extract of infected scallop larvae to uninfected scallop or oyster (*Crassostrea gigas*) larvae, demonstrating that it is able to infect both species. Detection of viral DNA in asymptomatic adult scallops by *in situ* hybridisation indicates that the herpesvirus may have been transmitted from adults to larvae. It is notable that, unlike most herpesviruses, this virus has a wide host range reflected by its ability to infect several species of marine bivalve.

Keywords: herpesvirus; scallop; *Pecten maximus*; OsHV-1; polymorphism; transmission; host range; bivalve

INTRODUCTION

Viruses morphologically similar to members of the family *Herpesviridae* have been identified in various marine bivalve species around the world (Renault, 1998). The first observation was reported in *Crassostrea virginica* adults (Farley *et al.*, 1972). Subsequently, herpes-like viruses were associated with high mortality rates in other cultivated oyster species including *Ostrea edulis* (Comps & Cochenec, 1993 ; Renault *et al.*, 2000a) and *C. gigas* in French (Nicolas *et al.*, 1992 ; Renault *et al.*, 1994a) and New Zealand (Hine *et al.*, 1992) hatcheries. Herpes-like viruses were also observed in haemocytes of *O. angasi* adults in Australia (Hine & Thorne, 1997) and in New Zealand in flat oysters, *Tiostrea chilensis* (Hine *et al.*, 1998). One of these viruses, isolated from moribund larval *C. gigas*, has been characterised as ostreid herpesvirus 1 (OsHV-1 ; Minson *et al.*, 2000), and its 207 kbp genome has been completely sequenced (A.J. Davison, unpublished data). Herpesviruses have also been characterised in non-ostreid bivalves, the European clam, *Ruditapes decussatus*, and Manila clam, *R. philippinarum* (Renault & Arzul, 2001 ; Renault *et al.*, 2001). A variant of OsHV-1 (hereafter termed OsHV-1var) was detected in Manila clam and Japanese oyster larvae which presented concomitant mortalities in 1997 in a French hatchery (Arzul *et al.*, 2001). OsHV-1 and OsHV-1var are representatives of a single viral species that may be the ubiquitous cause of observed herpesvirus infections of marine bivalves.

In September 2000, high mortality rates (100%) occurred among certain batches of scallop, *Pecten maximus*, larvae in a Breton commercial hatchery in France. Scallop aquaculture was developed locally in Brittany for restocking natural French environments, as this species had been seriously depleted in the 1980s owing to intense fishing pressure and unfavourable meteorological conditions. Breeding of larval and post-larval scallops is intensive, and is at risk from epizootic diseases, especially those caused by bacteria such as *Vibrio* species (Nicolas *et al.*, 1996). With the exception of small DNA-negative virus-like particles reported in *P. novaezelandiae* (Hine & Wesley, 1997), viral aetiology has not been linked to scallop mortalities. We describe here for the first time a herpesvirus that infects *P. maximus*, and show that it is ostensibly the same as OsHV-1var. Herpesviruses are usually detected in larvae and spat, but some reports concern adults (Farley *et al.*, 1972 ; Hine & Thorne, 1997), suggesting that bivalve herpesviruses are able to persist in adults without mortality (I. Arzul, unpublished data). Adult scallops belonging to the batch which produced infected larvae were analysed by *in situ* hybridisation and shown to be carrying the herpesvirus.

MATERIAL AND METHODS

Animals

Moribund *P. maximus* larvae, 7 or 10 days old, were collected in September 2000 from a hatchery located in Brittany (France). Before sampling, the larvae presented high mortality rates, which reached 100% in all batches by 10 days after fertilisation.

Ten adults from the same batch that produced the larvae were collected for *in situ* hybridisation analysis. These animals did not present any mortality or other symptoms of disease.

Intraspecies transmission

Seven day old *P. maximus* larvae from a Breton hatchery were placed at a concentration of 5 larvae per ml in 2 l flasks containing 0.22 µm filtered seawater. They were incubated at 18°C and fed with a mixture of three algae (*Pavlova lutheri*, *Isochrysis affinis galbana* and *Skeletonema costatum* or *Chaetoceros calcitrans*) at a concentration of 20 cells/µl for each species. Algae and antibiotic (see below) were added 3 times per week, and the culture water was completely renewed 3 times per week.

Three different assays, each replicated 3 times, were carried out using 7 day old healthy larvae. In assays 1 and 2, larvae were exposed to 1 ml of extract obtained by grinding 10⁵ moribund *P. maximus* larvae in 20 ml of sterile seawater using a Dounce homogenizer. In assay 1, the ground larvae were filtered through a 0.22 µm filter to remove bacterial contaminants. In assay 2, the ground larvae were not filtered. In assay 3 (the negative control), healthy larvae were maintained without adding ground larval extract. Chloramphenicol (4 mg/l) was included in all assays to reduce mortalities related to bacterial infections (Nicolas *et al.*, 1996).

Interspecies transmission

Moribund *P. maximus* larvae (400 mg) from a Breton hatchery were ground in 20 ml of seawater using a Dounce homogenizer and filtered through a 0.22 µm filter to remove bacterial contaminants. One µl of inoculum was sampled for PCR. Five 1 l flasks containing 3 day-old axenic *C. gigas* larvae were inoculated with 8 ml of ground larval extract (assays 1-5), and two 1 l flasks containing 3 day old axenic *C. gigas* larvae were inoculated with 8 ml of sterile seawater (assays 6-7). Three days after exposure, the larvae were recovered, concentrated and washed 3 times in filtered seawater by centrifugation (200 g for 15 min) as described by Arzul *et al.* (in press). One µl of seawater was collected for PCR analysis after each wash. After washing, larvae were treated for PCR analysis (Renault *et al.*, 2000b).

Transmission electron microscopy

Moribund larvae were fixed directly in 3% glutaraldehyde in 0.2 M sodium cacodylate (pH 7.2). Larval samples were then processed as described previously (Renault *et al.*, 1994a). Ultrathin sections were stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy (TEM) using a JEOL JEM 1200EX instrument operating at 80 kV.

PCR and sequence analysis

Larval samples were prepared by grinding frozen larvae in double distilled water, boiling and centrifuging (Renault *et al.*, 2000b). Supernatants were immediately diluted 10-fold in double distilled water and frozen at -20°C . Oligonucleotide primers were designed on the basis of the OsHV-1 genome sequence. Four primer pairs were used : Gp3 (5'-GGT TGT GGG TTT GGA AAT GT-3') and Gp4 (5'-GGC GTC CAA ACT CGA TTA AA-3') (698 bp product), B3 (5'-GTG GAG GTG GCT GTT GAA AT-3') and B4 (5'-ACT GGG ATC CGA CTG ACA AC-3') (207 bp product), C2 (5'-CTC TTT ACC ATG AAG ATA CCC ACC-3') and C4 (5'-GCA GTT GTG GTA TAC TCG AGA TTG-3') (352 bp product) and C2 and C6 (5'-GTG CAC GGC TTA CCA TTT TT-3') (710 bp product ; overlaps the C2/C4 product). The Gp locus (at approximately 134000 bp in the genome) encodes part of a putative glycoprotein, the B locus (146900 bp) encodes part of a putative inhibitor of apoptosis protein, and the C locus (present twice in the genome at 4500 and 178500 bp) encodes parts of two proteins of unknown functions (Arzul *et al.*, 2001). PCR reactions were carried out and the products analysed on 1% agarose gels as described previously (Renault *et al.*, 2000b). The positive control consisted of 10 ng/ μl OsHV-1 DNA extracted from purified virus particles (Le Deuff and Renault, 1999).

Gp3/Gp4 and C2/C4 PCR products derived from two moribund larval scallop samples were ligated into linearised pT-Adv vector (Clontech). Ligated DNA was transformed into competent *Escherichia coli* TOP10F'. Relevant portions of selected plasmids were sequenced using an ABI PRISM[®] sequencing kit (Perkin Elmer).

In situ hybridisation

In situ hybridisation was performed according to a previously described protocol (Renault & Lipart, 1998), except that 896 bp probes were produced by PCR using OsHV-1 DNA as template (0.1 ng/reaction), primers C1 (5'-TTC CCC TCG AGG TAG CTT TT-3') and C6 and digoxigenin-11-dUTP (Boehringer Mannheim) in a buffer containing 1.5 mM MgCl_2 . Detection was performed using an alkaline phosphatase-conjugated mouse IgG antibody against digoxigenin (1:500) incubated in NBT/BCIP (1:50). The slides were stained with Bismarck brown yellow, and mounted in Eukitt resin. Negative controls included samples processed without including digoxigenin-labelled probes or without antibody. An additional negative control consisted of samples hybridised with a digoxigenin-labelled probe specific to Spring Viraemia of Carp Virus (SVCV) kindly supplied by Dr R.M. Le Deuff (CEFAS, Weymouth, U.K). A positive control consisted of *C. gigas* juveniles shown to be infected with OsHV-1 by TEM and PCR.

RESULTS

ANALYSIS OF MORIBUND LARVAE

Detection of herpes-like virus particles by TEM

Viral particles were observed in the nuclei and cytoplasm of infected cells in the connective tissues of moribund *P. maximus* larvae collected at 7 or 10 days old. Nuclear particles were circular or polygonal in shape, 74-86 nm in diameter (Fig. 1). Some nuclear particles contained an electron-dense structure, and other particles appeared empty (Fig. 2). Naked nucleocapsids were also observed free in the cytoplasm of lysed infected cells (Fig. 3). Enveloped virions were detected in cytoplasmic vesicles (Fig. 4) and also in perinuclear spaces (Fig. 5). The occurrence of nuclear tubular structures 45 to 55 nm in diameter was also observed (Fig. 4). Extracellular particles were usually enveloped and measured approximately 110 nm in diameter. Infected cells showed hypertrophied nuclei and marginated chromatin densely packed around the nuclear envelope (Fig. 3). Degenerating and lysing infected nuclei and cells were observed frequently (Figs. 3 and 4).

Detection of oyster herpesvirus DNA by PCR analysis

Four samples (1-4) were analysed using primer pairs Gp3/Gp4, B3/B4 and C2/C4. Samples 1 and 2 consisted of 7 day old moribund *P. maximus* larvae and samples 3 and 4 consisted of 10 day old moribund larvae. The Gp3/Gp4 and B3/B4 primers amplified fragments of the expected sizes from the four larval samples and OsHV-1 DNA (Fig. 6a and 6b). The C2/C4 PCR products were about 180 bp smaller than those obtained with OsHV-1 DNA (Fig. 6c). No PCR products were detected in negative controls.

Analysis of viral DNA by sequencing

The Gp3/Gp4 and C2/C4 PCR products derived from larval samples 3 and 4 were sequenced. Two independent plasmids were processed for each product in order to rule out errors induced by PCR amplification. The sequences of each pair were identical. The sequences of the Gp3/Gp4 fragments from samples 3 and 4 were identical to each other and 99% identical to the OsHV-1 sequence (Fig. 7a). The nucleotide differences resulted in 7 amino acid residue substitutions, yielding 97% identity in the predicted protein sequences (Fig. 7b). The sequences of the C2/C4 fragments from samples 3 and 4 were identical to each other and to that of OsHV-1var (Arzul *et al.*, 2001), but different from that of OsHV-1 (Fig. 7c). Differences between OsHV-1 and OsHV-1var include several single nucleotide substitutions, insertions or deletions and, more notably, a deletion of 200 bp near the C2 primer site accompanied by an insertion of 27 bp. Arzul *et al.* (2001) registered the possibility that OsHV-1var lacks the region containing C2, and that this primer instead hybridizes fortuitously to a partially matched region.

TRANSMISSION ASSAYS OF VIRAL INFECTION

Intraspecies transmission of viral infection

In the 3 replicates of assays 1 and 2, larvae presented high mortality rates which reached 100% at 5 days after exposure to extract from moribund *P. maximus* larvae (Table I). Two

days after exposure, the mean mortality rates were 26.7 and 20% for the 3 replicates of assay 1 and assay 2, respectively (Table I). TEM revealed intracellular virus particles in these larvae. In the 3 replicates of assay 3, larvae not exposed to extract presented mean mortality rates of 2% at 2 days after exposure and 6% at 5 days after exposure (Table I). No viral particles were observed in these larvae by TEM.

Interspecies transmission of viral infection from scallop larvae to oyster larvae

Three days after exposure to extract from moribund *P. maximus* larvae (assays 1-5) or sterile seawater (assays 6-7), *C. gigas* larvae were recovered, washed three times and prepared for PCR. The initial inoculum, the three wash samples (S1-S3) and the larvae were analysed using primer pair C2/C6. Products from the inoculum appeared 180 bp smaller than those obtained with OsHV-1 DNA (Fig. 8). Samples S1-S3 in assays 6-7 did not yield products (Table II). For assays 1-5, the products from S1 and S2 were the same size as those from the inoculum (Table II and data not shown), and S3 did not yield products (Table II). Products from larvae in assays 1-5 were the same size as those from the inoculum (Fig. 8). Larvae from assays 6-7 and all the negative controls did not yield products (Fig. 8).

DETECTION OF OYSTER HERPESVIRUS DNA IN ADULT SCALLOPS BY *IN SITU* HYBRIDISATION

In order to investigate the presence of the herpesvirus in adult *P. maximus*, 10 specimens were processed for *in situ* hybridisation using digoxigenin-labelled DNA probes. Positive reactions characterised by a purple precipitate (in dark on Fig. 9) were detected in 7 animals. A similar signal was detected in infected spat, but no signal was detected when digoxigenin-labelled probe or antibody were omitted or when a digoxigenin-labelled probe specific to SVCV was used.

The abundance and intensity of the hybridisation signal in positive adult scallops varied between individuals and tissues. Labelling consisted of a small number of cells in the connective tissues of gills, gonad (Fig. 9), mantle and muscle. Staining was detected in both the cytoplasm and nuclei, and some stained cells exhibited anomalous chromatin patterns, including margination. In some cases, signal was also detected in epithelial cells of the gills.

DISCUSSION

Several descriptions of herpes-like virus infections in marine bivalves have been reported since 1972 in various species of oyster (Farley *et al.*, 1972 ; Hine *et al.*, 1992 ; Nicolas *et al.*, 1992 ; Comps & Cochenec, 1993 ; Renault *et al.*, 1994a ; Hine & Thorne, 1997 ; Hine, 1998 ; Renault *et al.*, 2000a) and clam (Renault & Arzul, 2001 ; Renault *et al.*, 2001). We now describe for the first time a herpesvirus infecting a species of scallop, *P. maximus*, in association with high larval mortality rates.

The virus particles observed by TEM in moribund *P. maximus* larvae collected from a commercial hatchery resemble those of herpesviruses of higher and lower vertebrates in morphology, virogenesis and size range (Roizman, 1982 ; Roizman, 1990 ; Roizman & Baines, 1991). These general characteristics of virus particles are similar to those documented previously in other bivalve species (Renault *et al.*, 1994b ; Renault *et al.*, 2001 ; Hine & Thorne, 1997 ; Hine *et al.*, 1998). A variety of morphological forms corresponding to replicating virus was noted in intranuclear and intracytoplasmic locations. Two classes of nucleocapsid were observed. One had an electron-dense core and corresponds to DNA-containing capsids, and the other lacked the core. Enveloped capsids (virions) were observed in perinuclear spaces, cytoplasmic vesicles and extracellular locations. Virus replication was associated with various cellular lesions, including cell lysis. Thus, the herpesvirus exhibits all the microscopic features of productive replication in scallop larvae. This is in contrast to another virus (infectious pancreatic necrosis virus), which has been described as being sequestered in the digestive tissues of *P. maximus* without replicating and being progressively transmitted through food chains to brown trout (Mortensen, 1993 ; Mortensen *et al.*, 1998).

The high mortality rate (100%) associated with detection of virus particles 5 days after exposure of healthy *P. maximus* larvae to infected larval extract fulfils Koch's postulates. Low mortality rates (6%) were observed 5 days after exposure of healthy larvae to sterile water, and no virus particles were detected. These results demonstrate that the herpesvirus induces larval mortalities and can be transmitted horizontally from infected to healthy scallop larvae. Variable mortality rates (0-62%) in larvae at 2 days after infection were probably due to variation in the quantity of infectious virus particles present in the inocula.

Infected larvae were analysed by PCR and DNA sequencing to assess whether the scallop herpesvirus is similar to OsHV-1. The ability to generate Gp3/4 and B3/4 PCR products of the expected sizes using primers based on the OsHV-1 sequence indicates that the scallop virus is very similar to OsHV-1. However, the sequence of the Gp3/4 product shows that the scallop virus is closely related, but not identical, to OsHV-1. Unusually, each of the 7 nucleotide substitutions is predicted to result in an amino acid substitution. The C2/C4 product was the same size as that for OsHV-1var (Arzul *et al.*, 2001) and 180 bp smaller than that for OsHV-1, and identical in sequence to that of OsHV-1var. Thus, it appears that OsHV-1var, which has been shown previously to infect oyster and clam larvae, also infects scallop larvae. It is not known whether OsHV-1 can infect scallop larvae.

The original source of OsHV-1var in the Breton hatchery, in which *P. maximus* is the only species bred, is unknown. However, the observations that this virus can infect several species in the field and that the scallop virus could be transmitted to *C. gigas* larvae under laboratory conditions are entirely consistent with acquisition from another bivalve species. A possible role for vertical transmission from adults to larvae is supported by the detection of viral sequences in asymptomatic scallops from the same batch of adults that produced the larvae. It

is notable that the connective tissue of the gonad was among the regions that contained infected cells. Vertical transmission has also been implicated for OsHV-1 in *C. gigas* (Le Deuff *et al.*, 1996), and viral proteins and DNA have been detected in healthy adult specimens of this species (I. Arzul, unpublished data).

This report extends the number of marine bivalve species that can be infected by OsHV-1var and adds to the pathogens already faced by *P. maximus* in commercial aquaculture. It highlights the risks of breeding different species in the same hatchery and indicates the importance of exercising strict precautionary measures to prevent the spread of herpesvirus infections between monocultures. It also stimulates further speculation that OsHV-1 and OsHV-1var are derived from a parental, and perhaps benign, bivalve herpesvirus that has been selected for virulence and wide host range by the practices of modern shellfish farming (Arzul *et al.*, 2001).

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Table I. Observed mortalities and transmission electron microscopy (TEM) examination of *Pecten maximus* larvae in the context of intraspecies transmission assays. Assay 1 : exposition of healthy larvae to a 0.22 μm filtered suspect larval extract. Assay 2 : exposition of healthy larvae to a not filtered suspect larval extract. Assay 3 : negative control assay, exposition of healthy larvae to sterile sea water.

Assay	Replicate	Mortality (%) [#]		TEM [†]
		2 days after exposure	5 days after exposure	
1	A	42	100	+
	B	5	100	
	C	33	100	
2	A	5	100	+
	B	62	100	
	C	0	100	
3	A	3	6	-
	B	2	6	
	C	1	6	

[#] Cumulative mortality of scallop larvae after exposure to moribund larval scallop extract or sterile seawater.

[†] +, intracellular herpesvirus particles detected ; -, herpesvirus particles not detected.

Table II. PCR analysis using the primer pair C2/C6 of the samples collected in the context of interspecies transmission assays. S1, S2 and S3 correspond to samples collected at each *Crassostrea gigas* larvae wash. Larvae correspond to the pellet obtained at the end of the three washes.

Assay	PCR analysis [#]			
	S1 : First wash	S2 : Second wash	S3 : Third wash	Larvae
1	+	+	-	+
2	+	+	-	+
3	+	+	-	+
4	+	+	-	+
5	+	+	-	+
6	-	-	-	-
7	-	-	-	-

[#] +, PCR products similar in size to those from the inoculum.

-, PCR products not obtained

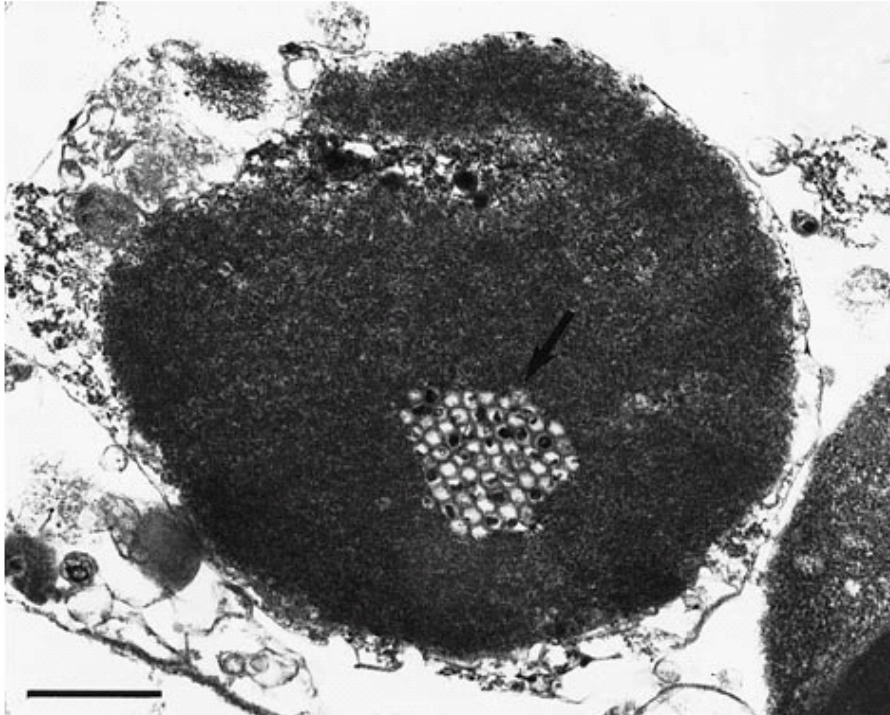


Figure 1. Infected lysed cell showing intranuclear capsids and nucleocapsids (arrow) in condensed nucleus. Bar : 500nm.

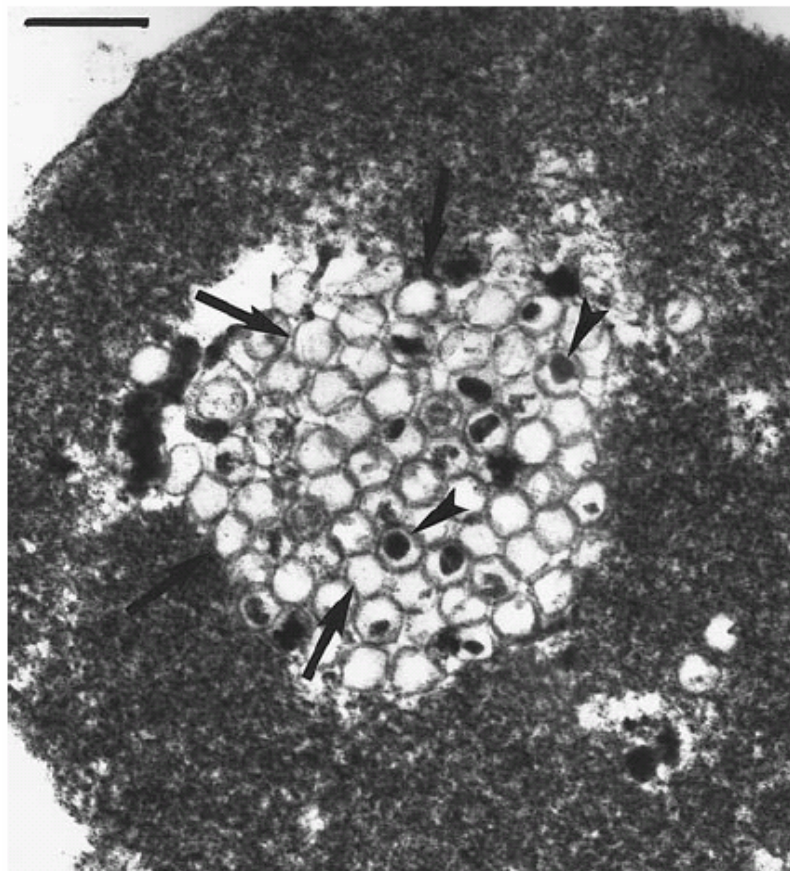


Figure 2. Infected nucleus containing capsids (arrows) and nucleocapsids with pleomorphic cores (arrowheads). Bar : 200nm.

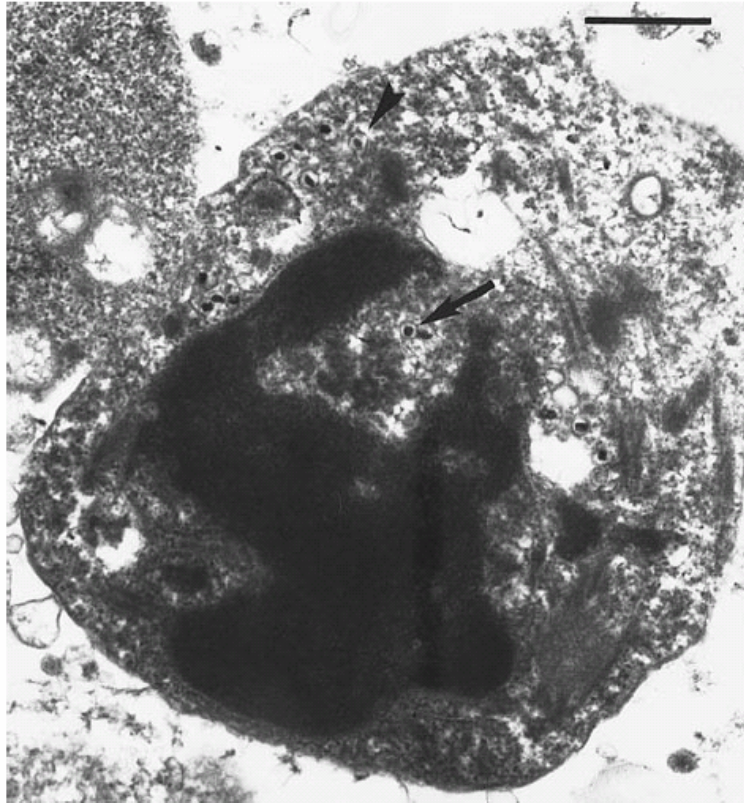


Figure 3. Lysed cell showing intranuclear (arrow) and intracytoplasmic virions (arrowhead).
Bar : 500nm.

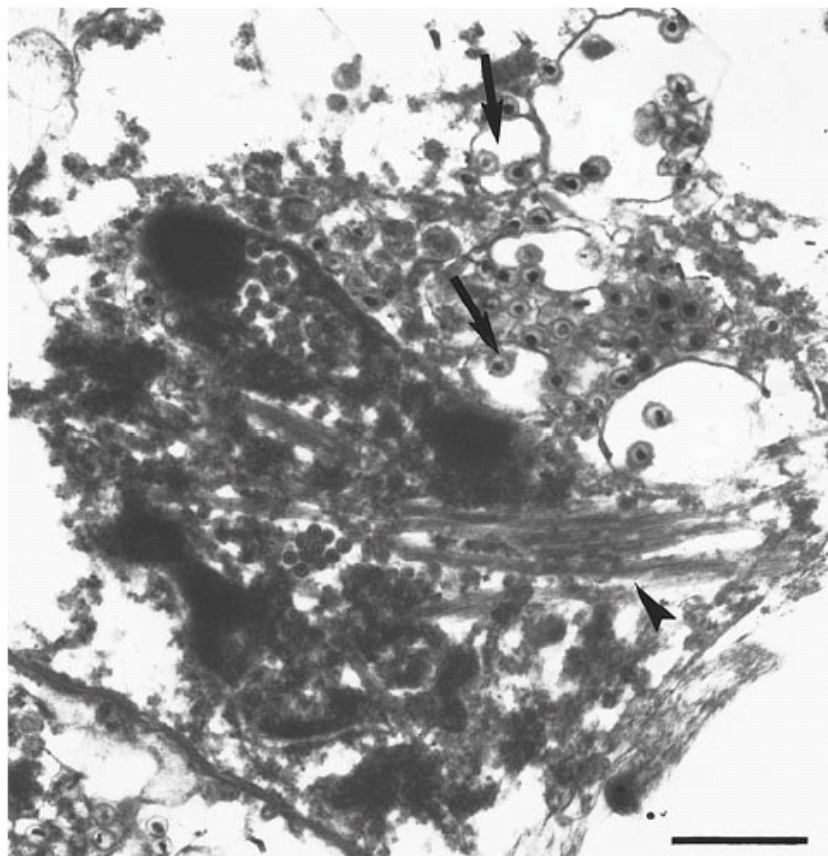


Figure 4. Infected cell showing enveloped virions in cytoplasmic vesicles (arrows) and intranuclear tubular structures (arrowhead). Bar : 500nm.

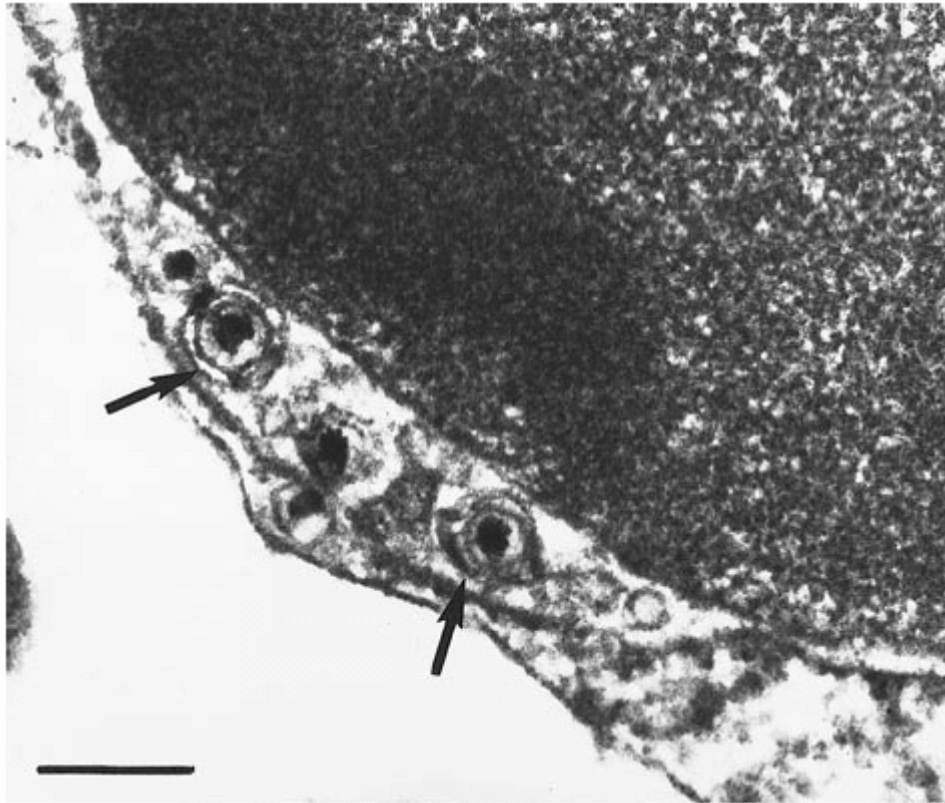


Figure 5. Enveloped virions (arrows) showing a longitudinal section of the toroidal core within perinuclear space. Bar : 200 nm.



Figure 6. PCR analysis of moribund scallop larvae using primer pairs (a) Gp3/Gp4, (b) B3/B4 and (c) C2/C4. 1-4 : samples of moribund scallop larvae. N : negative control (distilled water). P : positive control (OsHV-1 DNA). L : 100 bp ladder (Eurogentec).

a

S ggttgtggggtttggaaatgtAGATTTGAGCACTGTAAACACCCATGCCAGCAAAATTGGC
R ggttgtggggtttggaaatgtAGATTTGAGCACTGTAAACACCCATGCCAGCAAAATTGGC

S AGAAATATAAACCCCTAGATTCATGGTCGGTGTTTATACAAACGATAGCAATAAGTTAATC
R AGAAATATAAACCCCTAGATTCATGGTCGGTGTTTATACAAACGATAGCAATAAGTTAATC

S GAGGATGATATATACGGCCGTTACACAGATTCAGAATCTGCCGGTGTATGAGAAAAATGT
R GAGGATGATATATACAGCCGTTACACAGATTCAGAATCTGCCGGTGTATGAGAAAAATGT

S AATTTAAACGAGGTTAAACCACTCCCCAGGAAGATTGCATTCAGCCCTTTTGCACCAAA
R AATTTAAACGAGGTTAAACCACTCCCCAGGAAGATTGCATTCAGCCCTTTTGCACCAAA

S GGAACAGTGTATGGAAATAACCTCGTTTATGGAAGTAGATTACGACGTTTCAGTAGGAGC
R GGAACAGTGTATGGAAATAACCTCGTTTATGGAAGTAGATTACGATGTTTCAGTAGGACC

S AGGTGTTTCACAGAGAAGTAGAACCGTTCCTCAATCAGTCCCTTGGTATATACCATCTGGA
R AGGTGTTTCACAGAGAAGTAGAACCGTTCCTCAATCAGTCCCTTGGTATATACCATCTGGA

S TTTACAGGAAACCGGTTTATGTACCTGGATAATAGACTGGGATATCTTTTGGGATTAGAC
R TTTACAGGAAAACAGTTTATGTACCTGGATAATAGACTGGGATATCTTTTGGGATTAGAC

S CTTACCACGGCTATTTTTTAAATATACCCCAATTGTTGTTCGGACATATAGTAAGTGAATAC
R CTTACCACGGCTATTTTTTAAATATACCCCAATTGTTGTTCGGACATATAGTAAGTGAATAC

S CTGACGGGAATCATGAACTATAAGCGTCTCAGTGTGAGGAAAGGACCGAATATAGACATG
R CTGACGGGAATCATGAACTATAAGCGTCTCAGTGTGAGGAAAGGACCGAATATAGACATG

S CGAGGTATTATAGGTGGAGAAATCAAATGATATTGATAAGAACTACAGAAAGATGTTG
R CGAGGTATTATAGGTGGAGAAATCAAATGATATTGATAAGAACTACAGAAAGATGTTG

S GACATGAGTGGATTTACACTTTTGC CGGCGAATGGATGTTATGTCACAGTGATAAAAATTC
R GACATGAGTGGATTTACACTTTTGC CGGCGAATGGATGTTATGTCACAGTGATAAAAATTC

S ATCGGGGATAAACGCGTTttttaatcgagtttggacgcc
R ATCGGGGATAAACGCGTTttttaatcgagtttggacgcc

b

S GCGFGNVDLSTVNTHASKIGRNINPRFMVGVYTND SNKLI EDDIYGRYTDSE SAGVMRKC
R GCGFGNVDLSTVNTHASKIGRNINPRFMVGVYTND SNKLI EDDIYSR YTDSE SAGVMRKC

S NLNEVKTTTPQEDCIQPFCTKGT VYGNNLVYGSRLRRFSR SRC SQR SRTV P Q SVPWYIPSG
R NLNEVKTTTPQEDCIQPFCTKGT VYGNNLVYGSRLRCFSR TRC SQR SRTV P Q SVPWYIPSG

S FTGNRFMYLDNRLGYLLGLDLTTAIFKYTP IVVGHIVSEYLTGIMNYKRLSVRKGP NIDM
R FTGKQFMYLDNRLGYLLGLDLTTAIFKYTP IVVGHIVSEYLTGIMNYKRLSVRKGP NIDM

S RGIIGGEIKMILIRNYRKMLDMSGFTLLPANGCYVTVIKFIGDKRVFN RVWT
R RGIIGGEIKMILIRNYRKMLDMSGFTPLPVNGCYVTVIKFIGDKRVFN RVWT

Figure 7. Comparison of (a) nucleotide and (b) protein sequence of the Gp3-Gp4 fragment from scallop larvae (S) and reference OsHV-1 DNA (R). Nucleotide or amino acid substitutions are underlined in the S sequence. Primer sequences are in lower case.

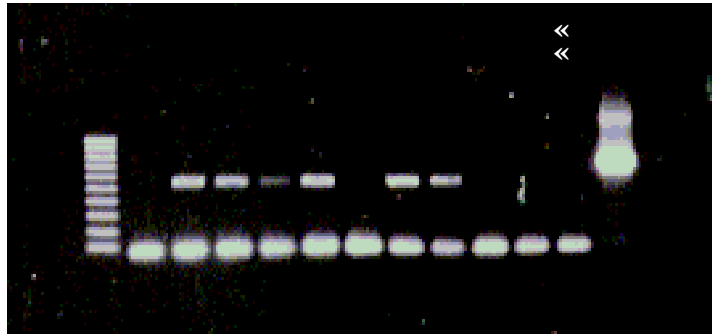


Figure 8. PCR analysis of samples subjected to interspecies transmission assays using primer pair C2/C6. I : inoculum of moribund scallop larval extract. 1-5 : *C. gigas* larvae exposed to the inoculum. 6-7 : *C. gigas* larvae exposed to sterile water. A-C : negative controls (distilled water). P : positive control (OsHV-1 DNA). L : 100 bp ladder (Eurogentec). The upper inverted comma indicates the positive control. The lower inverted comma indicates PCR products from samples.

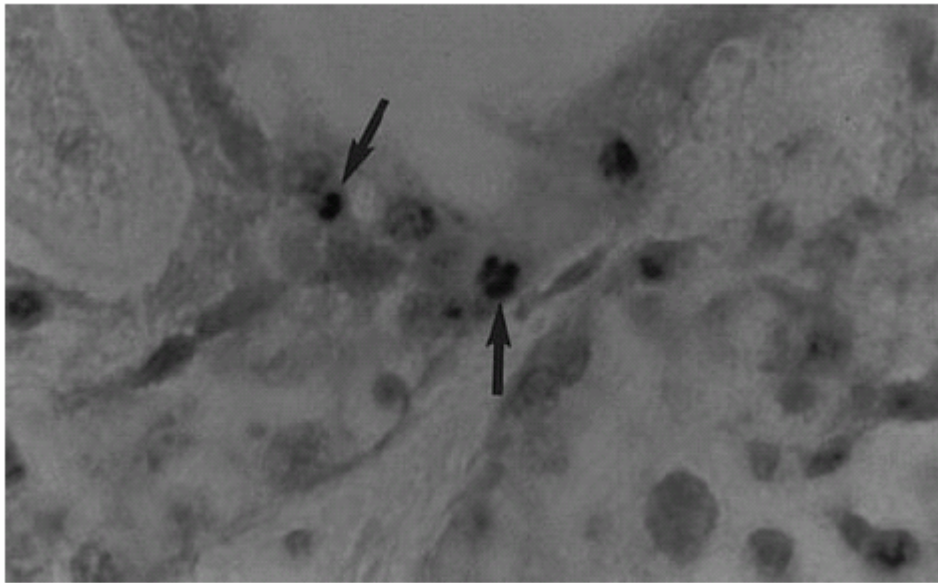


Figure 9. Positive cells characterised by a nuclear dark staining in gonad connective tissues from adult scallop (arrows).