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Viral gametocytic hypertrophy of the Pacific oyster *Crassostrea gigas* in Ireland

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ABSTRACT: Viral gametocytic hypertrophy (VGH) was detected during an investigation of mortalities in Pacific oysters *Crassostrea gigas* from 2 separate Irish production sites. The basophilic inclusions were observed in the gonad tissue of oysters sampled in August and October 2007. The oysters involved did not show any macroscopic disease signs. Transmission electron microscopy demonstrated the presence of viral particles in these intranuclear inclusions. The particles were small, non-enveloped, icosahedral and approximately 50 nm in diameter, and thus had characteristics similar to the *Papillomaviridae* and *Polyomaviridae* families. No host defence reaction was observed. The viral particles described here appear to be similar to those described in *C. virginica* from the USA and Canada and to those described in *C. gigas* from Korea and France.

KEY WORDS: *Crassostrea gigas* · Viral gametocytic hypertrophy · *Papillomaviridae* · *Polyomaviridae* · Pacific oyster · Gonad

INTRODUCTION

Numerous viruses can infect molluscs, and mortalities have been reported in different bivalve species associated with the presence of viruses belonging to various families (Elston 1997, Renault & Novoa 2004). Viruses described in bivalves have included members of the families *Herpesviridae*, *Reoviridae*, *Picornaviridae*, *Retroviridae*, *Birnaviridae*, *Iridoviridae* and *Papovaviridae*. The family *Papovaviridae* originally comprised the 2 genera *Papillomavirus* and *Polyomavirus*, but they are now considered as 2 separate families *Papillomaviridae* and *Polyomaviridae* (Van Regenmortel et al. 2000). These 2 families share morphological characteristics: viruses are non-enveloped, icosahedral and approximately 40 to 55 nm in diameter (Garcia et al. 2006).

Farley (1985) observed viral gametocytic hypertrophy (VGH) in hypertrophied cells of gonad tubules of *Crassostrea virginica* sampled in various US states but extensively in Maine. He described non-enveloped, icosahedral viral particles 50 to 55 nm in diameter in the maturing and mature cells. He also reported on histologically similar lesions seen in *C. gigas* and *Ostrea lurida* from Korea, Japan, Oregon and Washington and similar lesions in *C. rhizophorae* from Puerto Rico. Similar viral particles have also been described from *C. virginica* from the east coast of North America (Sparks 1985), the Gulf of Mexico (Winstead et al. 1998, Winstead & Courtney 2003) and Atlantic Canada (McGladdery & Stephenson 1994). Bower et al. (1994) reported the presence of viral particles with massive gamete hypertrophy in *C. virginica* from Atlantic Canada and the eastern United States; infection rates were generally low and there was no indication of associated mortalities.

Basophilic inclusions associated with VGH were described in the gonad tissue of *Crassostrea gigas* in southern Korea and, from electron microscopic observations, appeared to match the size range described for the polyomaviruses (Choi et al. 2004). Viral particles with characteristics similar to the *Papillomaviridae* and *Polyomaviridae* families have also been reported in *C. gigas* in France (Garcia et al. 2006). Moss et al. (2007) observed VGH histologically in the gonad of wild *C. hongkongensis* during a survey of Asian oysters for pathogens. Watermann et al. (2008) observed VGH in the hypertrophied gametocytes of *C. gigas* during investigations in to the health condition of these oysters along the East Frisian coast of Germany.

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The actual impact of papilloma-like and polyoma-like viruses on their hosts has not been fully assessed (Garcia et al. 2006). Neither is it clearly understood whether one or more viruses are involved in these gonad conditions. In 2007, we observed VGH in *Crassostrea gigas* gonad tissue sampled from 2 separate production sites in Ireland. We reprocessed the wax-embedded oyster gonad tissue for electron microscopy and describe the ultrastructure of the viral particles observed in these infected oysters.

MATERIALS AND METHODS

From August to October 2007, following reports of increased levels of mortalities, a total of 77 market-sized *Crassostrea gigas* were collected from 2 separate production sites in Ireland: Site A (County Kerry) and Site B (County Donegal) (Fig. 1).

Histological examination. Oyster tissue fixed in 10% v/v formalin solution was processed for routine histology. Sections were cut at 2 µm and stained with haematoxylin and eosin (H&E).

Ultrastructural examination. When inclusion bodies were observed during light microscopy, the wax-embedded oyster tissue containing the inclusion was reprocessed for transmission electron microscopy (TEM) as follows. With the H&E-stained section as a visual guide, the portion of wax-embedded tissue with the inclusion was removed with a scalpel from the wax block and dewaxed overnight in 2 changes of xylene with agitation. Following rehydration, the tissue was then placed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 to 5 h, rinsed again in 0.1 M cacodylate buffer and finally post-fixed in 1% OsO_4 for 2 h. After dehydration through graded alcohols, the tissues were infiltrated with a 1:1 solution of Agar low viscosity resin and 50% ethanol with agitation for 1 h, followed by 100% resin for 2 h minimum. Tissues were embedded in resin and cured at 60°C for 2 to 3 d. Semi-thin sections were stained with 1% toluidine blue and ultra-thin sections were stained with uranyl acetate and lead citrate. Ultra-thin sections were viewed using a Hitachi H-7500 transmission electron microscope at 75kV.

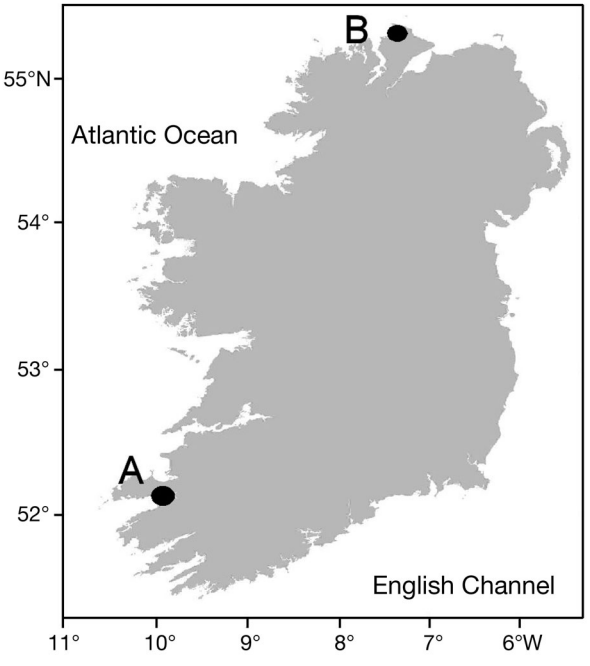

Fig. 1. Sample locations of infected *Crassostrea gigas* in Ireland collected between August and October 2007. Site A: County Kerry; Site B: County Donegal

RESULTS

No gross clinical disease signs were observed in the *Crassostrea gigas* collected from Site A (County Kerry) or Site B (County Donegal) between August and October 2007. In H&E-stained sections, basophilic inclusions were observed in hypertrophied nuclei in 2 of 53 oysters sampled from Site A during August and October, and in 1 of 24 oysters sampled from Site B in August. Infected maturing and mature ovocytes showed hypertrophied nuclei with perinuclear condensed nuclear material (Fig. 2).

There was no haemocytic infiltration or other host tissue reaction observed associated with the infection. TEM of reprocessed wax-embedded tissue containing the basophilic inclusions demonstrated that the granular inclusions consisted of a homogeneous amalga-

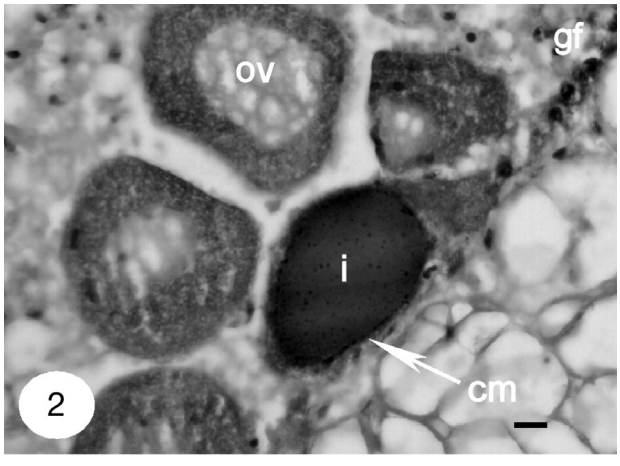

Fig. 2. *Crassostrea gigas.* Basophilic intranuclear inclusion in a gonad follicle of the oyster. Light micrograph of oyster gonad follicle (gf). Inclusion (i) and perinuclear condensed material (cm, arrow) in the nucleus of an ovocyte (ov). H&E staining. Scale bar = 10 µm

mation of viral particles. The nuclear membrane of the infected ovocyte was normal and peripherally displaced chromatin could be observed (Fig. 3). The viral particles were approximately 45 to 50 nm in diameter and non-enveloped (Fig. 4). They were 5- or 6-sided, suggesting an icosahedral symmetry (Fig. 5). Under TEM the viral particles from both sites appeared to be similar.

During the sampling period, 8 aquaculture sites experienced mortalities in Site A and cumulative mortalities ranged from 10 to 40%. In Site B, 4 operators noted mortalities of approximately 30%. From a total of 77 oysters examined, only 3 female oysters were found to have basophilic inclusions, with the number of infected cells ranging from 3 to 14 per section.

Based on the reprocessed TEM material, although of reduced quality, these viral particles appear similar to those described by Winstead & Courtney (2003), Choi et al. (2004) and Garcia et al. (2006).

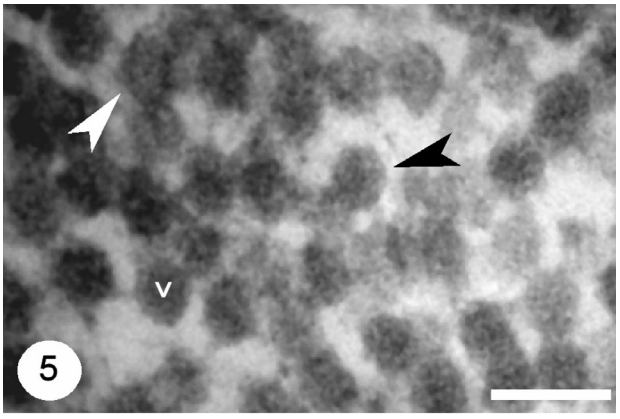

Fig. 5. *Crassostrea gigas.* Intranuclear 5-sided (white arrowhead) and 6-sided (black arrowhead) viral particles (v) in an ovocyte. Scale bar = 100 nm

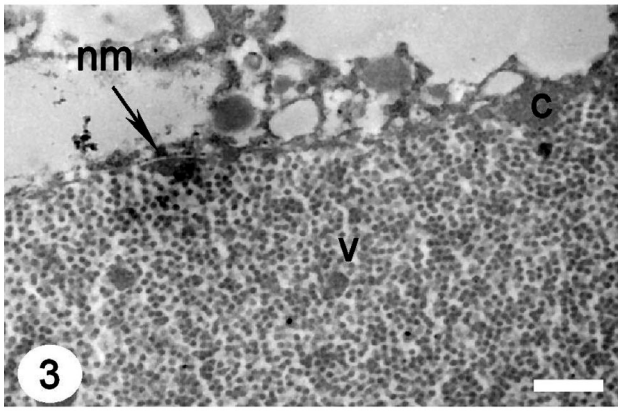

Fig. 3. *Crassostrea gigas.* Ultrathin section of inclusion body, showing intranuclear viral particles (v) in an ovocyte with a normal nuclear membrane (nm, arrow) and chromatin mass (c). Scale bar = 500 nm

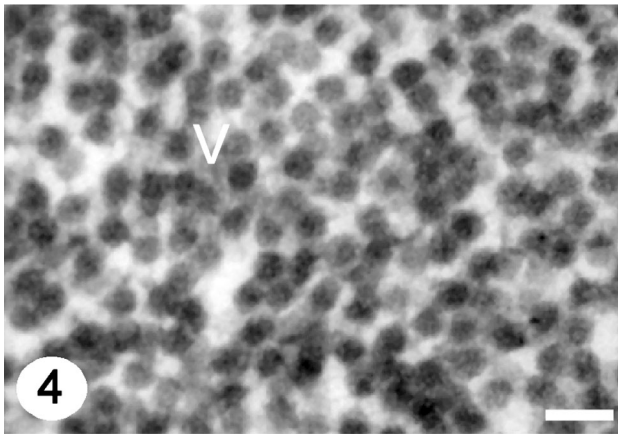

Fig. 4. *Crassostrea gigas.* Ultrathin section of inclusion body showing details of viral particles (v), which are non-enveloped, icosahedral and 45 to 50 nm in diameter. Scale bar = 100 nm

DISCUSSION

Farley (1976, 1985) described a papova-like virus in hypertrophied gametocytes of the eastern oyster *Crassostrea virginica*; since then other authors have reported similar conditions in various oyster species in North America, Asia and Europe (McGladdery & Stephenson 1994, Elston 1997, Choi et al. 2004, Garcia et al. 2006). This is the first report of VGH in *C. gigas* in Ireland. Observations at the ultrastructural level in the present study show that the basophilic inclusions seen in histology are in fact large masses of viral particles in the hypertrophied nuclei of gonad tissue. The size and symmetry of these particles suggest similarity to the *Papillomaviridae* and *Polyomaviridae* families (Van Regenmortel et al. 2000); however, further studies would be required to formally assign the viral particles to these families.

Papilloma- and papova-like viruses have been described from various bivalve species (Elston 1997). However, without the availability of molluscan cell lines, none of these viruses have been isolated and characterised, and insufficient knowledge is available from histopathological and ultrastructural studies alone to discriminate between these viruses described from various parts of the world.

Although VGH is readily detected in maturing gametes, it is more difficult to detect in non-mature oysters (Garcia et al. 2006). A maximum infection level of 350 cells (average of 4 infected cells per section) was reported in *Crassostrea virginica* by Farley (1985), who also noted that female oysters were more often in-

fected. Garcia et al. (2006) observed up to 16 infected cells per section in *C. gigas*, and also noted that *C. gigas* male and female oysters were equally affected by VGH. However, Watermann et al. (2008) observed up to 20 infected cells per section in *C. gigas*, and reported that male oysters were more commonly infected. These author also noted that, even though there had been previous surveys carried out along the East Frisian coast in 2003 and 2004, VGH had not been detected, as was also the case in France before 2001 (Garcia et al. 2006). In the present study we observed between 3 and 14 infected cells per section in 3 female oysters; however, the number of oysters examined is too low to establish infection rate or infection intensity.

In common with other studies (Choi et al. 2004, Garcia et al. 2006), no haemocytic reaction was observed in the present study, suggesting limited health implications for the infected oysters. However, Garcia et al. (2006) comment that gamete viability and consequently oyster fecundity could be altered by VGH. In the present study the stocks examined were experiencing mortalities, but the low number of oysters detected with VGH and the lack of any clinical disease signs would suggest that the observed virus particles were unlikely to be causing the mortalities. Since 1993, oyster mortalities have been repeatedly experienced during the late summer months in many of the Irish *Crassostrea gigas* production areas, without the identification of any linked pathogen or pathogens. The mortalities experienced here fit this pattern.

So far no serious manifestations are known for this virus, but the possibility exists for oncogenic transformation (Harshbarger et al. 1979, Farley 1985, Van Regenmortel et al. 2000, Watermann et al. 2008). Potential interspecific cross-infection may produce disease in other, possibly more susceptible, hosts. This would have significant implications, particularly in the case of the introduction of non-native species (Munn 2006, Watermann et al. 2008).

Virus-like particles have been identified in many species of bivalve molluscs (Renault & Novoa 2004), although proof of aetiology and study of pathogenesis is often lacking (Munn 2006). Viruses may be found in molluscs already debilitated by disease or by other stress factors (Montes et al. 2001). On the other hand, viruses may be observed simply due to bioaccumulation, and their presence may not necessarily imply disease. Infectious disease is a complex interaction between the agent, the host and the environment. It is also necessary to distinguish between viral infection and actual disease manifestation. By definition, a virus is infective for its particular host(s), but may have varying effects on different life stages of the host and may be more virulent for different species (Elston 1997). At present, diagnosis of viral disease is by light microscopy followed by confirmation using TEM. The lack of molluscan cell lines has impeded the advancement of bivalve virology, but recently the use of molecular tools has become more widespread (Munn 2006). Cultivation trials, followed by the physical isolation of viruses and the use of genetic probes and other molecular tools, can assist in advancing this field.

Viral diseases are of concern in intensively reared molluscs because no specific chemotherapies or vaccines are available. A better understanding of the virus and virus–host interaction is required for disease control in aquaculture and for reducing the transmission of viral diseases between cultured and natural populations of bivalve molluscs. Advancement in the field of molluscan virology will require increased application of physical isolation methods, the development of continuous molluscan cell lines and the use of molecular tools and should be the focus of further studies.

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