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Viral gametocytic hypertrophy caused by a papova-like virus infection in the Pacific oyster *Crassostrea gigas* in Korea

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ABSTRACT: During a routine survey of the Pacific oyster *Crassostrea gigas* in Tongyoung (previously Chungmu) on the southern coast of Korea, basophilic inclusions were observed in the gonadal tissues. They were detected from March to May at a prevalence rate of 3.3 to 7.1%. The inclusion bodies were Feulgen-positive and stained orange-red with phloxine tartrazine. Electron microscopic observation revealed non-enveloped, icosahedral particles 40 to 45 nm in diameter. These morphological characteristics resemble those of papova virus-like inclusions previously described from Pacific and eastern (American) oysters *C. virginica* in North America. Although many mitochondrial bodies and intact sperm cells were observed around the inclusion body, no host reaction, such as hemocytic infiltration, was detected.

KEY WORDS: Pacific oyster · *Crassostrea gigas* · Viral gametocytic hypertrophy · Papova-like virus · Seminal gland

INTRODUCTION

Tongyoung (previously Chungmu) is an important area for oyster culture in Korea; however, production has declined significantly over the last decade. One of the reasons for this decline is mortality due to infectious disease. Marteilioidosis, caused by the protistan parasite *Marteilioides chungmuensis*, is one of the main oyster diseases (Comps et al. 1987), but other diseases may also be contributing to these mortalities and have yet to be properly investigated.

Papova-like viruses have been reported from the labial palp epithelial of gold-lipped pearl oysters in northern Australia (Norton et al. 1993) and from the gonadal epithelia of *Crassostrea virginica* in Canada (McGladdery & Stephenson 1994) and the USA (Meyers 1981, Farley 1985, Winstead et al. 1998, Winstead & Courtney 2003), as well as from other bivalve species (Elston 1997). During a recent health survey of cultured oysters from the Tongyoung area, papova-like virus was observed in gonadal tissues. This is the first confirmed report of this virus in *C. gigas* from Korea.

MATERIALS AND METHODS

Pacific oysters *Crassostrea gigas* 1 to 2 yr old, cultured at Tongyoung on the southern coast of Korea, were collected from October 2001 to November 2002. From 6 sites (Fig. 1), ~30 oysters were randomly collected each month (total of 2340 oysters). Individual lengths were recorded along with gross and microscopic observations. For histological analysis, tissue samples were fixed in Carson's fixative solution (OIE 2000) and Davidson's fixative solution (Howard & Smith 1983).

Histological examination. Oyster tissues fixed in Davidson's solution were processed for paraffin-

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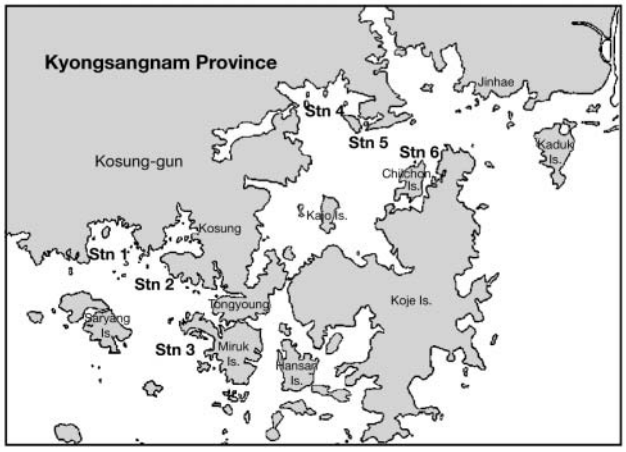


Fig. 1. Sampling stations in Tongyoung on the southern coast of Korea

embedding and cut into 4 µm thick sections. Some sections were stained with Harris's hematoxylin and eosin (H&E), some with Feulgen stain and some with phloxine tartrazine, for bright field light microscopy (Olympus Vanox AHBS3) examination.

Ultrastructural examination. Samples showing inclusion bodies within the gonadal epithelia were selected for processing for transmission electron microscopy. Tissue specimens from the same oysters, preserved in Carson's fixative solution were rinsed in 0.2 M cacodylate buffer at pH 7.2 for 48 h at 4°C before post-fixing in 2.5% glutaraldehyde solution, and 1% osmium tetroxide. After dehydration through graded alcohols, the tissues were embedded in Epon resin compound (Ouken, Japan). Hardening was carried out at 35°C for 12 h, 45°C for 12 h, and 60°C for 48 h. Semi-thin sections (200 nm) were stained with toluidine blue, and ultra-thin sections (60 nm) were stained with uranyl acetate and lead citrate. Ultra-thin sections were examined with a JEOL 1200 EX-2 transmission electron microscope (TEM) at 80 kV.

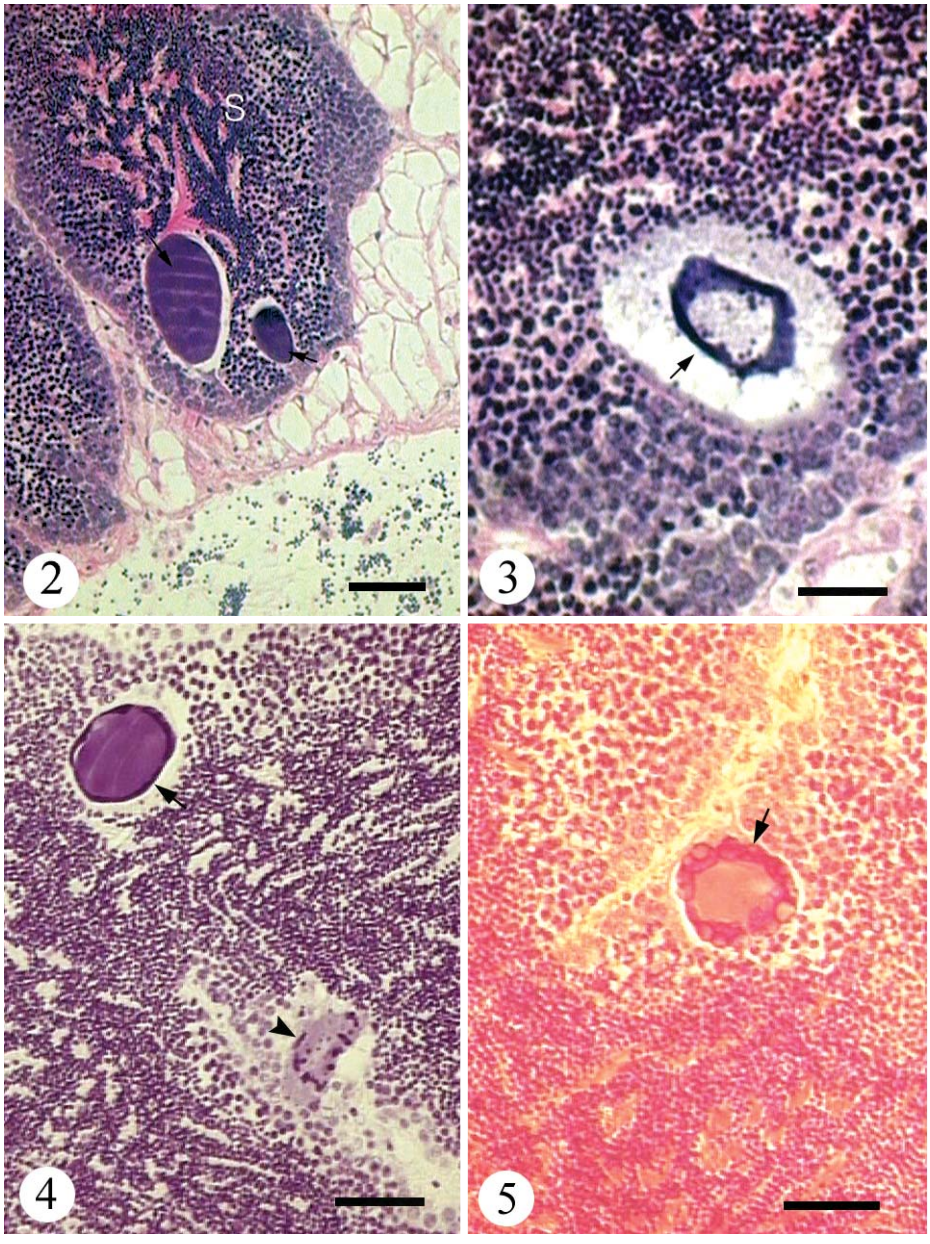
RESULTS

Basophilic inclusions in gonoducts were observed in specimens from all sites except Stn 4. Infections were observed from March to May at prevalences of 3.3 to 7.1%. Prevalence at Stn 3 was higher than at the other sites (Table 1). The heaviest infection included 62 inclusion bodies in a single tissue section; however, no host reaction was observed. The longest axis of the inclusion bodies varied from 15 to 60 µm, and the shape was oval to spherical. Some inclusion bodies had dense staining margins (Figs. 2 & 3). Feulgen staining confirmed the presence of dense nucleic acid (Fig. 4), and orange-red staining with phloxine tartrazine (Fig. 5) demonstrated a deoxyribonucleic acid (DNA) composition. Advanced infections led to disruption of the nuclear membrane and putative release of the viral particles (Fig. 4, arrowhead).

Non-enveloped, icosahedral viral particles, 40 to 45 nm in diameter, filled the inclusion bodies (Figs. 6 & 7). These morphological characteristics resemble those of *Papovaviridae*. The chromophilic margins observed by light microscopy were identified as peripherally displaced chromatin. Many mitochondria were arranged around the intranuclear inclusion bodies. Spermatozoa were scattered around the inclusion bodies (Fig. 8), and few free virus particles were observed around inclusion bodies (Fig. 9).

Table 1. *Crassostrea gigas*. Prevalence of viral gametocytic heterotrophy (VGH) in oysters sampled at 6 stations in Tongyoung area, southern coast of Korea. Data are no. infected/no. sampled (% prevalence). –: not detected; ns: not sampled; shaded values: infection period

Date	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Stn 6
2001						
October	–/30	–/30	–/30	–/30	–/30	–/30
November	–/30	–/30	–/30	–/30	–/30	–/30
December	–/30	–/30	–/30	–/30	–/30	–/30
2002						
January	–/30	–/25	–/30	–/25	–/25	–/25
February	–/30	–/28	–/30	–/30	–/25	–/25
March	–/28	–/30	1/30(3.33)	–/30	1/30 (3.33)	1/20 (5)
April	1/30 (3.33)	1/30 (3.33)	2/28 (7.14)	–/27	–/22	1/29 (3.45)
May	1/30 (3.33)	2/30 (6.67)	1/30 (3.33)	–/30	1/30 (3.33)	–/30
June	–/30	–/30	ns	–/30	–/24	ns
July	–/29	–/30	ns	–/26	–/18	ns
August	–/28	–/30	ns	–/22	–/25	ns
October	–/30	–/30	–/30	–/30	–/30	–/30
November	–/30	–/27	–/30	–/28	–/21	–/24



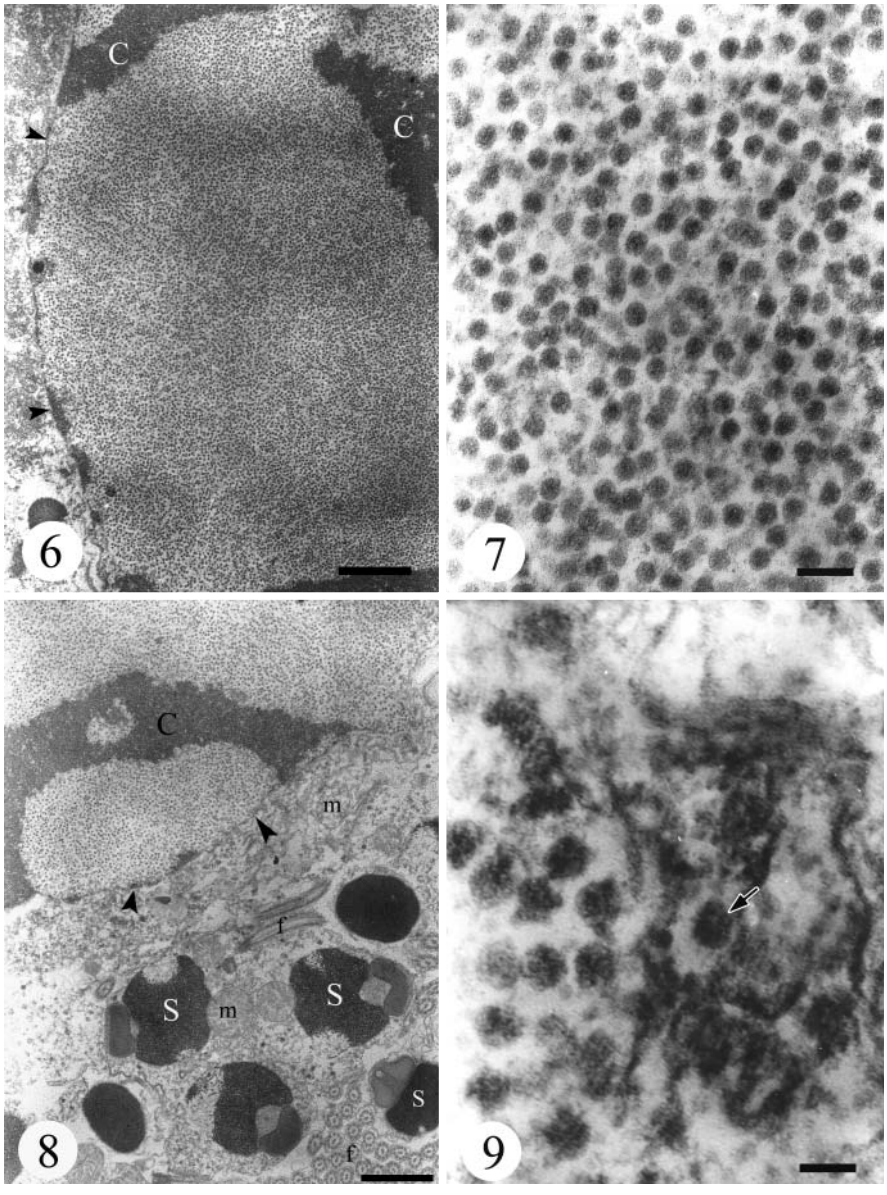
Figs. 2–5. *Crassostrea gigas*. Fig. 2. Basophilic homogeneous inclusion bodies (arrows) are among germ cells in reproductive gland of male; surrounding tissue lacks necrotic cells (H&E) (scale bar = 35 µm). Fig. 3. Inclusion body is organized into a ring-like structure of condensed chromatin; unstained regions outside and inside the inclusion body (H&E) (scale bar = 20 µm). Fig. 4. Feulgen-positive inclusion body (arrow) and other structure (arrowhead) with indistinct outline and spotted chromatin debris (Feulgen reaction) (scale bar = 25 µm). Fig. 5. Inclusion body (arrow) with several nuclei at its margin and stained orange-red (phloxine tartrazine) (scale bar = 25 µm)

DISCUSSION

Based on ultrastructural morphological characteristics and staining properties, these viruses appear consistent with those previously described as papova-like (Dimmock & Primrose 1994, Ackermann & Berthiaume 1995, p. 63–64) from gonoduct lesions in oysters from North America (*Crassostrea gigas*, *C. virginica*, *Ostrea lurida*, *O. edulis*) and Australia (*C. commercialis*) (Farley 1976, 1978, 1985, McGladdery & Stephenson 1994, Winstead et al. 1998, Winstead & Courtney 2003). Infections of labial palp epithelia in *Pinctada maxima* (gold-lipped pearl oyster) were determined to be caused by a similar virus (Norton et al. 1993).

In vertebrates, *Papovaviridae* include the papillomoviruses responsible for warts, papillomas and related skin disorders. The family also includes the polyomaviruses responsible for polio disease.

The viruses detected in the present study appear to match the size range described for the polyomaviruses; however, those of *Crassostrea virginica* appear to be more closely associated with the papillomaviruses (McGladdery & Stephenson 1994, McGladdery 1999). No papilloma-like lesions were detected; however,



Figs. 6–9. *Crassostrea gigas*. Fig. 6. Transmission electron micrograph of inclusion body, filled with viral particles, arrowheads: edge of inclusion body; C: condensed chromatin; scale bar = 1 µm. Fig. 7. Details of viral particles, non-enveloped, icosahedral and 40 to 45 nm in diameter; scale bar = 100 nm. Fig. 8. Transmission electron micrograph of inclusion body and surrounding tissue; arrowheads: edge of inclusion body; C: condensed chromatin; m: mitochondria; S: head of spermatozoa; f: flagella of spermatozoa; scale bar = 1 µm. Fig. 9. Released virus particle (arrow) outside inclusion body; scale bar = 50 nm

viral proliferation causes significant hypertrophy and compression of gonoduct space available for gametogenesis. Single cells appear to be infected, with no evidence in the current study (or previously published observations of similar infections) of neoplastic proliferation of infected cells/tissues. No adverse effect was noted on oyster health; however, if the viral infections were to become more intense, the effect on fecundity would need to be assessed.

Infections were noted in March, April and May, with no clear link to environmental factors over this period, except possibly to increased water temperature. Further study is required to clarify the seasonal dynamics and relation to the normal oyster gametogenesis cycle.

In Korea, reproduction of oysters depends mostly on the collection of natural spat. Viral gametocytic hypertrophy (VGH) would affect the reproduction of oysters, but it would be difficult to quantify this effect. Most cultured oysters collected in spring are harvested before the second summer to avoid summer mortality. VGH may become a problem in Korea and also worldwide. Thus, its impact should be the subject of further studies.

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