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Haplosporidiosis of the Pacific Oyster, *Crassostrea gigas*

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Haplosporidan parasites were observed in 10/100 spat and 1/171 adult Pacific oysters, *Crassostrea gigas*, reared in Matsushima Bay, Japan. Eight of the infected spat contained mild to severe plasmodial infections. The multinucleated plasmodia were 6–12 μm × 7–15 μm and were associated with an infiltration of hemocytes that occurred throughout the vesicular connective tissues of all infected oysters. Two oysters, one adult and one spat, contained advanced sporogonic infections. These were characterized by the presence of sporocysts and immature and mature operculated spores that measured 5.6–6.0 μm × 6.0–8.0 μm and were found exclusively within the digestive tubule epithelium. Electron microscopic examination revealed that mature spores contained a hinged operculum, striated and layered wall, spherule, single nucleus, and haplosporosome formative regions. Parasite morphology and infection pattern closely resemble that of *Haplosporidium nelsoni*, a pathogen of American oysters (*C. virginica*).

KEY WORDS: Pacific oyster, *Crassostrea gigas;* haplosporidan; plasmodia; spores; digestive tubule epithelium; vesicular connective tissue.

INTRODUCTION

Haplosporidan parasites are known to infect a variety of bivalve hosts including the American oyster, *Crassostrea virginica* (Farley, 1965; Krantz et al., 1972; Andrews, 1976), the Olympia oyster, *Ostrea lurida* (Mix and Sprague, 1974), the European flat oyster, *O. edulis* (van Banning, 1977), the California sea mussel, *Mytilus californianus* (Taylor, 1966), and the Pacific oyster, *C. gigas* (Rosenfield et al., 1966; Katkansky and Warner, 1970; Kern, 1976). Two species, *Haplosporidium nelsoni* and *H. costalis,* are associated with significant mortality of *C. virginica* along the east coast of the United States (Andrews, 1976). Infections of bivalves with other haplosporidan species are not usually associated with mortality (Mix and Sprague, 1974), although Katkansky and Warner (1970) observed a heavy haplosporidan infection within the vesicular connective tissues (VCT) of a moribund Pacific oyster. Haplosporidans in Pacific oysters from several countries, including Korea (Kern, 1976), Taiwan (Rosenfield et al., 1966), and the Netherlands (van Banning, 1977, 1979), have been detected during routine surveys for bivalve parasites.

We report the discovery of haplosporidan infections in adult and juvenile (spat) Pacific oysters from Matsushima Bay, Japan during routine histological examinations.

MATERIALS AND METHODS

Gross examination. Sixty adult and 40 juvenile (spat on cultch) *C. gigas* were collected from Matsushima Bay, Japan on November 14, 1989 and were sent to the California Department of Fish and Game, Fish Disease Laboratory for a routine health examination prior to importation into California. The adults were examined for condition and gross signs of disease. Two additional shipments of oysters were collected from the same location in Matsushima Bay, Japan. Sixty adult and 40 spat oysters were collected January 10, 1990 and 51 adult and 120 spat oysters were collected February 8, 1990.

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Light microscopy. Two 4-mm cross sections, one section including the heart and a second section posterior to the labial palps, were removed from each oyster. Tissues were fixed for 48 hr in Davidson's solution (Shaw and Battle, 1957) and were processed for routine paraffin embedding. Spat were scraped off cultch, fixed in Davidson's solution, decalcified with formic acid-sodium citrate (Luna, 1968), and processed for routine paraffin embedding. Five-micrometer sections were stained with hematoxylin and eosin (Luna, 1968). Selected sections were also stained by the Ziehl–Neelsen acid-fast (Difco Laboratories, Detroit, Michigan) and Wolbachs' Giemsa methods (Luna, 1968). Parasites were measured from stained sections of affected oyster tissues.

Electron microscopy. Tissues from one oyster embedded in paraffin were examined by electron microscopy. The tissues were deparaffinized, rehydrated to 100% water through a graded series of alcohol, fixed in 2.5% glutaraldehyde in 0.1 *M* Karnovsky buffer (pH 7.4) for 1 hr at 22°C, and post-fixed in 1% osmium tetroxide in 0.1 *M* Karnovsky buffer for 1 hr at 4°C. The tissues were rinsed twice in buffer, dehydrated through a graded series of acetone, infiltrated, and embedded in epoxy resin. Thin sections (10–20 nm) were stained with uranyl acetate and lead citrate prior to examination with a Phillips EM 400 electron microscope.

RESULTS

Gross and microscopic pathology. The adult oysters from the first shipment appeared healthy and gravid. Adults from the second and third shipments appeared either gravid and fat or were in early stages of gonad resorption and appeared slightly watery. No external signs of disease were observed. Histological examination of stained sections from the first shipment revealed the presence of an advanced haplosporidan infection within the digestive tubule epithelium and VCT of one adult (1–2 years) and one juvenile oyster (<6-month-old spat on cultch). The infection was accompanied by a diffuse infiltration of host hemocytes into the VCT surrounding the digestive tubules. The infected adult oyster contained multinucleated plasmodial stages within the VCT surrounding the digestive tissues that measured 6.6 × 9.4 μm (N = 3; SD = 1.7 × 1.9) with a range of 6–8 μm × 7–10 μm. Numerous sporogonic stages and spores in various stages of development were observed exclusively within the digestive tubule epithelium. The mature spores measured 5.1 × 6.7 μm (N = 9; SD = 0.4 × 0.6) in maximum dimensions, ranged in size from 5.6 to 6 μm × 6 to 8 μm, and stained acid-fast by the Ziehl–Neelsen method and bright blue by Wolbachs' Giemsa method. Sporocysts located within the digestive tubule epithelium contained up to 48 spores with an average of 24 spores (N = 11; SD = 11.6), measured 27.6 μm (N = 9; SD = 9.4), and ranged in size from 20.0 to 46.0 μm in maximum diameter (Fig. 1). Development of the sporocyst resulted in lysis of digestive tubule epithelial cells. Mature and immature spores were found free within the lumina of affected tubules. The infected spat oyster contained numerous mature and immature spores which had replaced all of the normal digestive tubule epithelial tissues.

No parasites were observed in adult oysters examined in January or February. In contrast, 5/40 spat in the January sample had mild infections of multinucleated plasmodia within the VCT that elicited a diffuse infiltration of hemocytes. Four of approximately 100 spat examined in February contained haplosporidan infections. Two of the spat had severe systemic infections with multinucleated plasmodia throughout the VCT that measured 7.9 × 10.1 μm (N = 24; SD = 1.6 × 1) in average size which ranged from 6 to 12 μm × 7–15 μm (Fig. 1). A strong inflammatory infiltrate of hemocytes and necrosis of host tissues surrounding the parasites characterized the systemic infections. Another 2 spat had mild infections

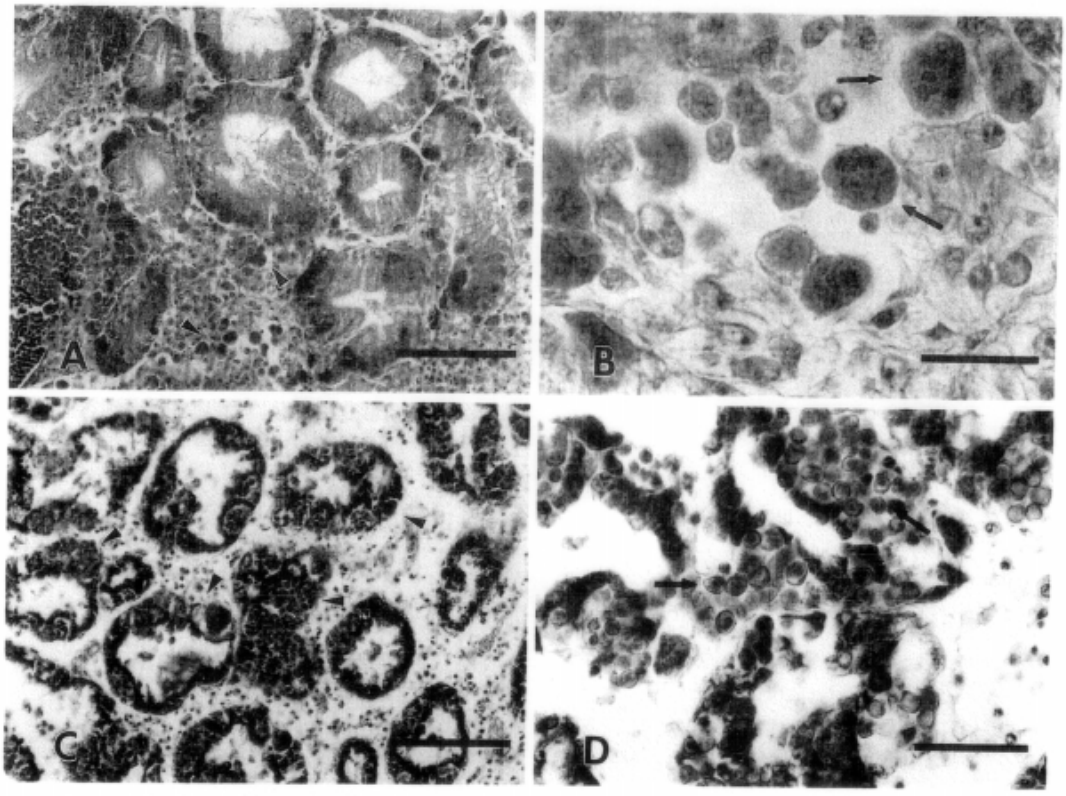

FIG. 1. Haplosporidan parasites found within vesicular connective tissues (VCT) and digestive tubules of Pacific oysters, *Crassostrea gigas*, from Japan. (A) Spat oyster with an infiltration of hemocytes surrounding multinucleated plasmodia (arrows) within VCT. Bar = 200 μm. (B) Histological section showing the multinucleate and pleomorphic nature of the haplosporidan plasmodia (arrows). Bar = 19 μm. (C) Histological section through an adult Pacific oyster with an advanced sporogonic infection. Arrows indicate sporonts and mature spores within digestive tubule epithelium. Bar = 200 μm. (D) Arrows point to operculated mature spores which have destroyed adjacent digestive tubule epithelium. Bar = 21 μm.

within connective tissues consisting of only a few plasmodia and hemocytes. No sporogonic stages were observed in these infections.

Electron microscopy. Mature spores each possessed a hinged, operculated orifice and were surrounded by an epispore cytoplasm. The operculum originated from and extended beyond the wall of the spore. The spore wall appeared layered and contained striations perpendicular to the axis of the spore wall. Remnants of a spherule or golgi complex were observed directly beneath the operculum. Haplosporosome formative regions were observed surrounding the nucleus which was located posterior to the golgi complex (Fig. 2). No definite evidence of spore ornamentation was discerned in these samples.

DISCUSSION

The haplosporidan parasite of *C. gigas* from Matsushima Bay, Japan resembles *H. nelsoni* of *C. virginica* in tissue specificity, morphology, and size of plasmodial and sporogonic stages (Perkins, 1968; Kern, 1976). Infections with these two haplosporidans are characterized in early systemic phases by the presence of multinucleated plasmodia throughout the oyster's VCT (e.g., gill, gonad, and digestive tissues)

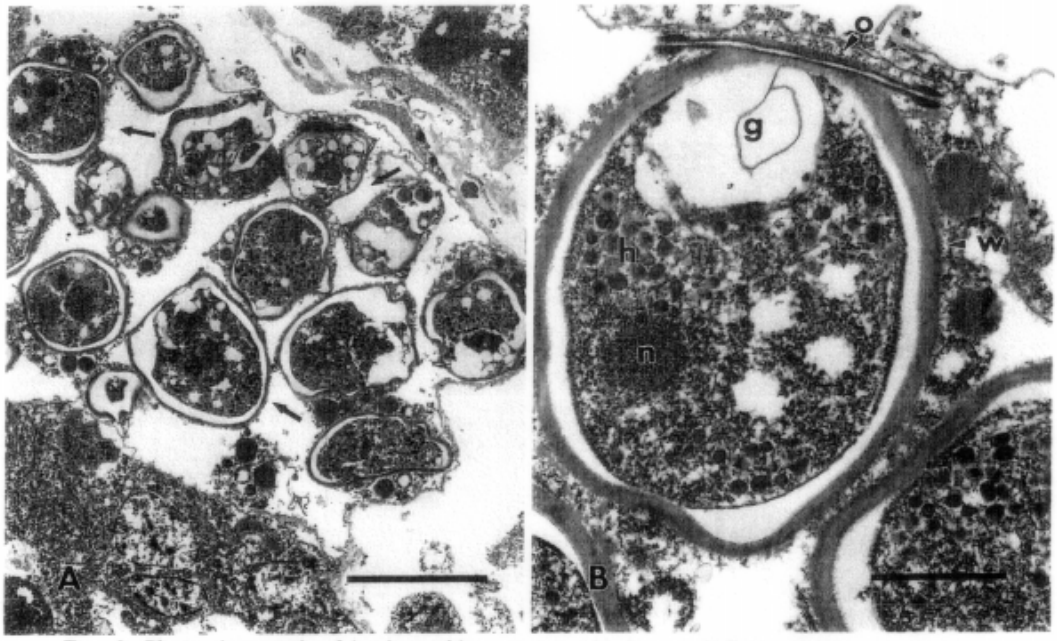

FIG. 2. Photomicrograph of haplosporidan spores. (A) Arrows indicate numerous spores surrounded by extraspore cytoplasm. Bar = 6.7 μm. (B) A mature spore showing a layered, striated wall (w), a hinged operculum (o), remnants of a spherule or golgi complex (g), a nucleus (n); and a haplosporosome formative region (h). Bar = 1.4 μm.

(Andrews, 1979). Plasmodial stages elicit a strong hemic response. In later infections, plasmodia may be observed within or adjacent to the epithelium of digestive tubules. Developing sporonts and sporocysts containing immature or mature spores are found exclusively within digestive tubule epithelia in advanced infections. Plasmodia and sporogonic stages in various stages of development may be present in the same oyster. Sporulation, therefore, appears to be asynchronous in contrast to infections with *H. costalis* in which synchronous sporulation occurs throughout the VCT (Andrews, 1979). Both *H. nelsoni* and the parasites observed in our study had a multinucleated plasmodial stage which ranged in size from approximately 5 to 15 μm and uninucleated spore stages of approximately 5–6.4 μm × 6–8.4 μm (van Banning, 1977; Andrews, 1979, Sprague, 1982).

Electron microscopic examination of parasitized Pacific oyster tissues revealed spores with similar morphology to those described by van Banning (1979) and McGovern and Burreson (1990) for various members of the Haplosporidiidae. These researchers relied upon accurate description of the nature and origin of spore ornamentation to distinguish between the genera *Haplosporidium* and *Minchinia* in the Haplosporidiidae. Members of the genus *Haplosporidium* contain spores ornamented with filaments that are extensions of the spore wall and persist after lysis of the epispore cytoplasm. Two species, *H. nelsoni* and *H. costalis,* contain spore wrappings that Perkins (1968, 1979) described as distinct structures that develop within the epispore cytoplasm and subsequently become attached to the spore wall as the epispore cytoplasm degrades. In contrast, members of the genus *Minchinia* are ornamented with tails that are extensions of epispore cytoplasm and form as a result of cytoplasmic degradation and concentration

of microtubule-like structures during spore maturation (McGovern and Burreson, 1990). These structures are never in contact with the spore wall and have been observed to be shed upon incubation for 7 days in sea water (McGovern and Burreson, 1990).

The inability to observe spore ornamentation in our samples may be due to several causes. The haplosporidan from Japanese *C. gigas* may lack ornamentation or the filaments or wrappings may have been destroyed due to the harsh treatment of the tissues during processing. Perkins (1988, 1989) suggested that operculated spores with extraspore extensions visible by light microscopy belong in the genus *Minchinia* and those without such easily observable filaments or tails belong in the genus *Haplosporidium*. Based on these data, the parasite observed in Pacific oysters from Japan appears to belong in the genus *Haplosporidium*. Electron microscopic examination of fresh material will be pursued to determine the presence and origin of spore ornamentation of this haplosporidan.

Whether this parasite causes mortality of Pacific oysters is not known. Oyster mortality has occurred annually in Japan since 1945 concurrent with the advent of the hanging method of oyster culture (Koganezawa, 1974). Mortality has been attributed to reproductive acceleration and physiological stress due to elevated water temperatures and nutrient levels (Imai et al., 1965; Tamate et al., 1965). No haplosporidans or other parasites were observed in conjunction with these oyster mortalities.

A majority of haplosporidan infections of Pacific oysters (Kern, 1976) and sporulated stages of *H. nelsoni* in American oysters (Andrews, 1976) have been observed in spat oysters. Whether the haplosporidan parasite of Pacific oysters is pathogenic for spat and/or adult oysters awaits further experimentation. Speciation of the *Haplosporidium* sp. observed in our study and its relationship to other members of the genus *Haplosporidium* will require further morphological, antigenic, and/or nucleic acid analysis of freshly attained parasites.

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