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Mortalities of Juvenile Shrimp, *Penaeus monodon*, Associated with *Penaeus monodon* Baculovirus, Cytoplasmic Reo-Like Virus, and Rickettsial and Bacterial Infections, from Malaysian Brackishwater Ponds

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Abstract

A disease syndrome from Malaysia in extensively reared *Penaeus monodon*, in which *P. monodon* baculovirus (MBV), a cytoplasmic reo-like virus, rickettsia and bacteria were identified pathologically, is described. Typical MBV lesions were apparent in hepatopancreases; the reo-like virus did not produce any light microscope-visible lesions. Rickettsial microcolonies, present in hypertrophied connective tissue cells throughout all shrimp tissues, were associated with hemocyte infiltrations, aggregations and granulomas with melanization and fibrocytes. The organs with the highest prevalence of rickettsia-associated lesions were the heart, hepatopancreas, Y-organ, gills and parietal hemocoele linings. The Gram-negative, rod-shaped bacteria were associated with tissue changes and had a tissue distribution typical of a penaeid bacterial septicemia. Histologically, the rickettsia appeared primarily responsible for the disease with secondary bacterial infections contributing to the mortalities seen. MBV and the reo-like virus may have facilitated the rickettsial infection or may have become patent in the weakened shrimp.

Introduction

Development of coastal fringe areas for brackishwater pond culture is a rapidly growing segment of the Malaysian aquaculture industry. In 1984, 600 t of penaeid shrimp, mostly *Penaeus monodon*, were produced from 674 ha of ponds (Abdul-Kadir 1986). It is estimated that, if 20% of all Malaysian mangrove forests are utilized, 114,000 ha would be available for further brackishwater pond development (Abdul-Kadir 1986). As in any new animal culture system, constraints on production can arise. Such has been the case in Malaysia with inappropriate imported aquaculture technologies needing modification and adaptation to local environmental and social systems.

High mortalities in extensively reared shrimp have been infrequently reported. It is more in the intensive culture systems where the development and transmission of infectious diseases is encouraged (Lightner 1983, 1985). The artificial environment of high stocking densities, inadequate nutrition, trauma and chemical pollutants found in intensive systems stresses the shrimp transforming a subclinical infection into an overt disease (Overstreet 1978). Extensive culture allows the shrimp access to natural foods to balance otherwise imperfect compounded feeds and reduces the interactions between individuals minimizing culture stress and disease; further, dead shrimp sink, making detection of disease in extensive culture difficult, with low prevalences often missed altogether.

Several pathogens of penaeids have been implicated in disease of juvenile and subadult populations in Southeast Asia. *Penaeus monodon* baculovirus (MBV) is a wide distribution and is known to be present in Malaysia (Lightner 1985). Bacterial infections of the cuticle (shell disease) and generalized septicemias have been recorded in the Philippines, Indonesia and Thailand (Primavera and Apud 1977; Nurdjana et al. 1978; Ruangpan 1978) and are considered to have a worldwide distributon (Johnson 1978; Lightner 1983). While it has been reported that the serious mycotic disease caused by *Fusarium solani* has a low incidence in pond-reared shrimp (Lightner 1977) and that *P. monodon* relatively resistant to infection (Lightner et al. 1979), Ruangpan (1978) reported an instance of 50% mortality in pond-reared *P. monodon* on one farm in Thailand. There is yet to be any record of *Fusarium* infection in other countries in the region. Both the studies of Primavera and Apud (1977) and Ruangpan (1978) referred to epicommensal fouling as a cause of disease in pond-reared *P. monodon*.

This paper reports an occurrence of clinical disease in extensively pond-reared *P. monodon* juveniles in Malaysia which involved a multiple infection with MBV, a reo-like virus, a rickettsia-like organism and bacteria.

Juvenile shrimp, *P. monodon,* were investigated from a farm which had a history of mortalities in the State of Johore, Peninsular Malaysia, consisting of 152 ha of earthen ponds, ranging in size from one to six hectares, developed from a mangrove forest area. Water was pumped from the surrounding estuary into a supply channel. Under gravity, water flowed to inlets, which consisted of concrete, screened monks. Ponds drained into a separate drainage channel and could be emptied or filled independently of all other ponds. Postlarvae (PL) for pond stocking were produced by the farm's hatchery from wild-caught spawners, usually originating from the State of Sabah, Malaysia. Rates from 5 PL m^{-2} to 10 PL m^{-2} of PL 18 stage were used for stocking. Monitoring of basic water quality parameters, including algal bloom density, was done three times a day. Dissolved oxygen concentrations were never below 5 mg l^{-1}. Paddle wheel aerators were used in the smaller ponds when necessary. Commercial pelleted shrimp feeds were used at rates of 4-12% of total shrimp biomass. An estimated 1-2% of each pond population originated from the wild.

Since the problem started, various management procedures had been implemented in an attempt to control and/or prevent mortalities. Changes in water management (e.g., exchange rates), source of feed, feeding rates, stocking rates, pond preparation and the use of a two-stage pond rearing method (nursery and growout ponds) did not affect the clinical syndrome observed. Mortalities occurred at any time of the year. All ponds were equally likely to have mortalities.

Detailed chemical analysis of the soil and water was carried out by the farm management. It was reported that while the soil contained significant amounts of some metals and had a pH of 5.76 to 6.58, the water characteristics were suitable for shrimp culture. There were no harmful metal levels and the water pH ranged from 6.93 to 7.4.

Materials and Methods

Diseased *P. monodon* juveniles from three different ponds were sampled for clinical examination. All were 2.5 months of age. Pond 1

(6 ha) had been having mortalities for one week. Mortalities had just started in the other two ponds, pond 2 (6 ha) and pond 3 (1 ha).

Specimens from each of the three ponds were injected and immersed in Davidson's fixative (Humason 1967). After 24 hours the fixed shrimp were transferred and stored in 10% phosphate-buffered formalin. Tissues were processed for routine histopathology and all sections stained with hemotoxylin and eosin (H&E), Brown and Brenn's modification of Gram's and Ziehl-Neelsen (ZN) stains. Selected sections were stained with periodic acid-Schiff stain (PAS) and Pinkerton's stain for rickettsia (Luna 1968). Tissue destined for electron microscopy was further trimmed, postfixed in 2.5% glutaraldehyde in 0.2M Sorensens phosphate buffer (pH 7.4) followed by 1% osmium tetroxide in phosphate buffer, dehydrated conventionally and embedded in resin. One-micron semi-thin sections were stained with 1% toluidine blue and thin sections with saturated uranyl acetate and lead citrate (Venable and Coggeshall 1965). The latter were examined using a Jeol-100C transmission electron microscope.

Results

Clinical History

Mortalities had been occurring over several production cycles, with the first mortalities always from seven to nine weeks post-stocking. Initially, lethargic shrimp were seen at the pond sides in early morning with a few individuals erratically swimming near the water surface during the day. Mortalities began three days after the first clinical signs were noted and continued over the next two or three weeks. The diseased shrimp were reported as presenting darkening of the gill filaments, empty foreguts, watery feces in the midgut and six to seven small whitish nodules, approximately 0.5 mm in diameter on the midgut wall. The affected shrimp always appeared small for their age; consequently production from the affected ponds was severely reduced. Only *P. monodon* was affected by the disease. Wild *P. merguiensis* and *P. indicus* within the ponds were unaffected and grew well.

Histopathology

A number of histopathological changes were apparent in shrimp from all ponds. The pathology observed appeared to relate to concurrent *Penaeus monodon* baculovirus (MBV), rickettsial and systemic bacterial infections. A reo-like virus infection was also apparent when hepatopancreatocytes were examined with electron microscopy. Lesions relating to this infection were not apparent histologically and the findings describing the reo-like virus are reported separately (Nash et al., in press).

Intranuclear MBV occlusions were relatively common in animals from all ponds, with an overall prevalence of 61% for all shrimp examined. Electron microscopy revealed the typical rod-shaped, singly enveloped virions and intranuclear viral occlusions. Hypertrophied hepatopancreatocyte nuclei, with one to seven refractile eosinophilic occlusions, were more obvious in the hepatopancreatic ducts. Hepatopancreatocytes of the distal tubule tips were less often infected with MBV, although "signet ring" nuclei, with dense perinuclear accumulations of basophilic material, were not uncommon. Infected epithelial cells of the stomach or midgut were not seen. Many of the hepatopancreatocytes with hypertrophied nuclei or intranuclear occlusions were undergoing hydropic degeneration, with large clear cytoplasmic vacuoles apparent. In those hepatopancreases with heavy infections, some tubules were necrotic and there was a sloughing of hepatopancreatocytes into tubule lumens. Of the sloughed cells, 5 to 20% had intranuclear occlusions. In all of the hepatopancreases examined, many normal hepatopancreatocytes remained. Even cells adjacent to infected cells were usually normal. No changes associated with MBV infection were observed in any other organ or tissue.

The most severe lesions, observed in shrimp from ponds 1 and 2, were associated with hypertrophied, round to oval cells which had peripherally displaced and flattened nuclei, and finely granular basophilic cytoplasm. These cells strongly resembled those which have been described previously as rickettsial microcolonies (Fig. 1). The majority measured 33 to 8 µm by 19 to 5 µm. Electron microscopy revealed rickettsia-like organisms within thin walled vacuoles (Fig. 2). The organisms were rod shaped with a size range of 690-770 by 17-23 nm. Rickettsial microcolonies were observed in the hepatopancreas, Y-organ, epidermis and underlying connective tissue, gill filaments and branches (from which the filaments arise), heart, tegmental glands, antennal glands, nervous tissue, connective

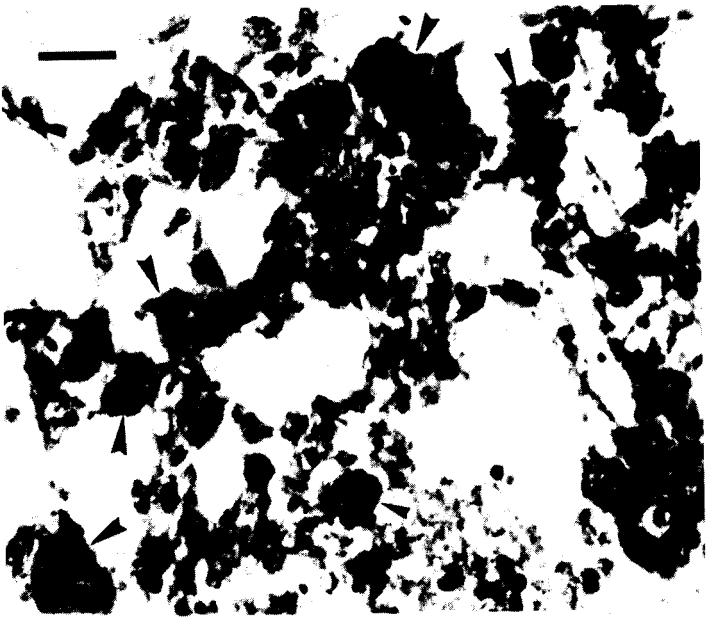

Fig. 1. Rickettsial microcolonies (arrowheads) within hypertrophied connective tissue cells of *P. monodon* Y-organ. H & E; bar = 20 μm.

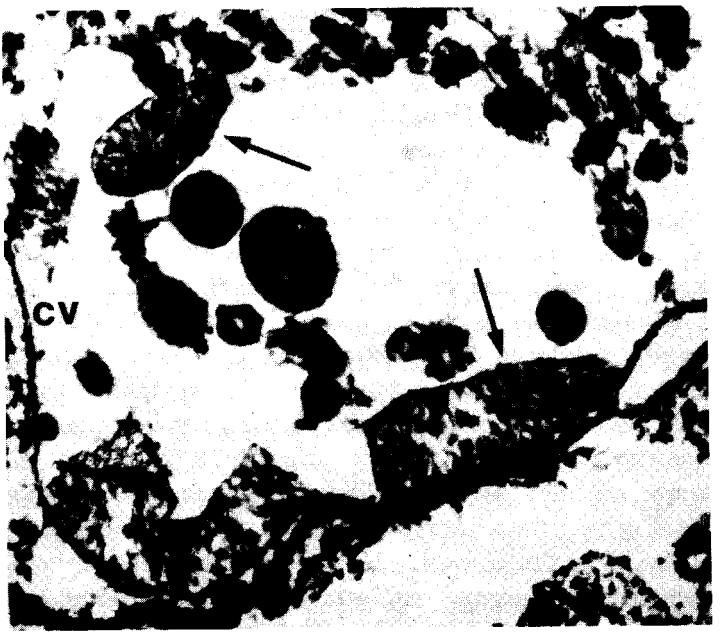

Fig. 2. Electron micrograph of rickettsial organisms (arrows) within a thin walled cytoplasmic vacuole (cv) in a heart hemocyte. Uranyl acetate and lead citrate stain, 2,000 x magnification.

tissue, outer layers of the foregut and midgut, gnathothoracic muscular myosepta, connective tissue lining of the ventral hemolymph sinus (near the origin of the periopods), dorsal hemocoele and in the parietal lining of the hemocoele surrounding the hepatopancreas.

In all of these tissues, there was often an associated area of degeneration and necrosis, hemocyte aggregations and granulomas (encapsulations). The most severe changes appeared in the Y-organ, where there was a total disruption of normal tissue structure (Fig. 3). Epithelial cells were degenerate and necrotic. Edematous separation of cell cords, with numerous scattered pyknotic and karyorrhectic nuclei, was present. Throughout this area were numerous rickettsial microcolonies. Under 1,000 x magnification, the majority of the fibrocytes, hemocytes, degenerating cells and epithelial cells contained small numbers of very short, weakly Gram-negative, intracytoplasmic rickettsia-like rods. The microcolonies and intracytoplasmic rods stained red with Pinkerton's rickettsial stain and were PAS-negative.

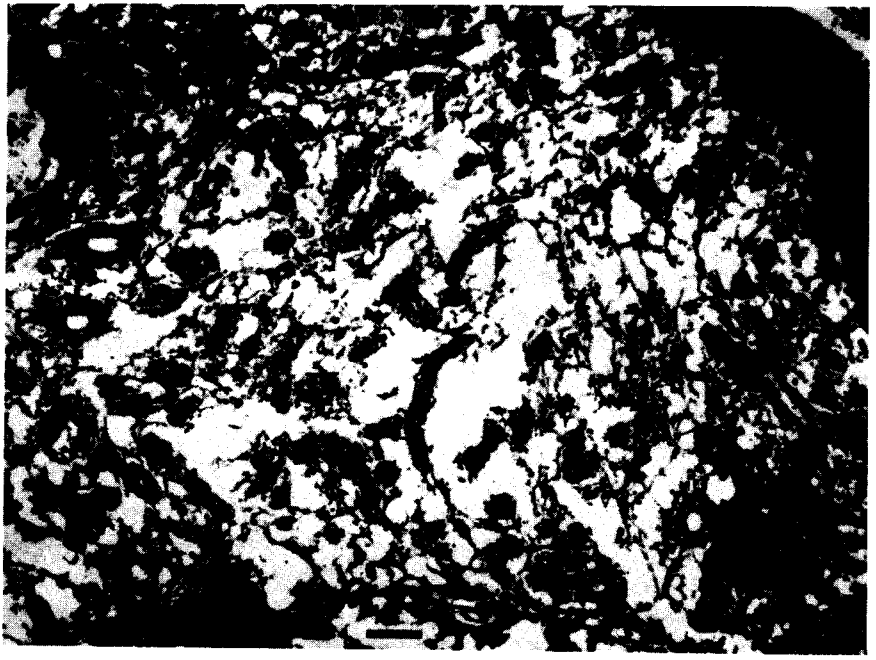

Fig. 3. Sclerotic Y-organ. Note only a few cell cords remain intact. H & E; bar = 100 μm.

Rickettsial microcolonies were observed as discrete single hypertrophied cells or in association with hemocytic aggregations. The aggregations varied in size and were particularly noticeable in the heart (Fig. 4a), peripheral hepatopancreas, intertubular tissue in the distal aspects of the hepatopancreas, in the parietal linings of the numerous hemolymph sinuses in the gnathothorax and in the gill branches. Less often aggregations were apparent in the outer connective tissue layer of the midgut (Fig. 4b) and in a number of other areas, but always associated with connective tissue.

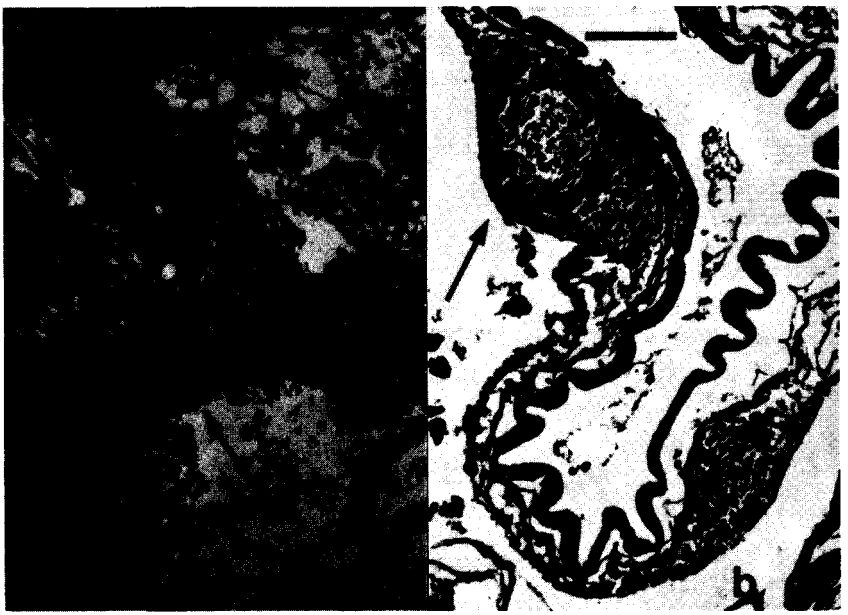

Fig. 4. a) Hemocyte aggregations (arrows) together with hypertrophied cells containing rickettsial microcolonies in the heart. H & E; bar = 200 μm. b) A hemocyte aggregation (arrow) in the connective tissue surrounding the hindgut. H & E; bar = 100 μm.

The structure of the aggregations was similar in all sites within the shrimp (Fig. 5). Numerous eosinophilic granulocytes and fibrocytes aggregated into a focal area often forming large nodules. Pyknotic nuclei and karyorrhectic nuclear material was present, scattered throughout the aggregation. The cellular arrangement of the aggregations was relatively loose with, particularly in the heart

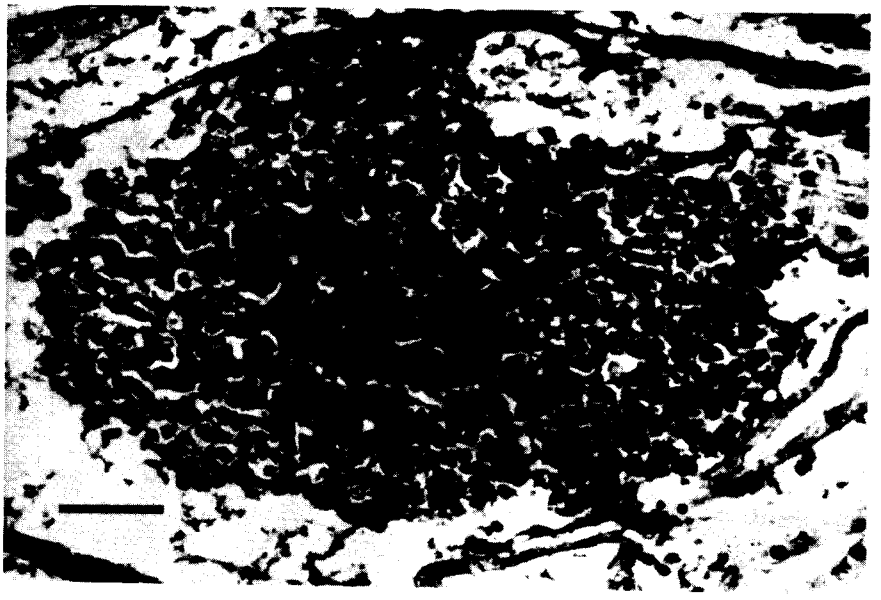

Fig. 5. A hemocyte aggregation. H & E; bar = 40 μm.

and hepatopancreas, many rickettsial microcolonies present within the collection of hemocytes and fibrocytes. Often the tissue immediately surrounding the aggregation was edematous. When the cellular aggregations were present in sections of the midgut the profile of the outer layers would bulge into the surrounding hemolymph sinus (Fig. 4b). In Gram-stained sections examined under 1,000 x magnification, the majority of the fibrocytes and many of the hemocytes present in the aggregation contained small numbers of weakly Gram-negative intracytoplasmic rickettsia-like rods.

Melanised granulomas, consisting of a very compact central zone of necrotic and degenerating cells, were frequently present in the peripheral area of the hepatopancreas and occasionally observed in the gill branches and gnathothoracic parietal lining tissues (Fig. 6a). If cytoplasm could be discerned in the central zone, it tended to be more eosinophilic than in normal cells. Bizarre shaped, pyknotic and karyorrhectic nuclei were present throughout this area. Surrounding the necrotic core was a compact zone of melanised hemocytes. Artifactual separation of the melanised layer and an outer zone of loosely arranged hemocytes, fibrocytes and rickettsial microcolonies was sometimes apparent. Again under high magnification, intracytoplasmic rickettsia-like rods were present in many of the cells

involved in the granuloma, including those in the central zone. From careful examination of the outer zone and the cellular aggregations, it appeared that the fibrocytic cells were the first to become infected as it was only in these cells where a few rickettsia-like rods could be seen within the cytoplasm of what were otherwise apparently normal cells, although this could not be confirmed with electron microscopy. In severe infections the granuloma outer zone would consist nearly completely of hypertrophied cells with intracytoplasmic rickettsial microcolonies. In no section did hepatopancreatocytes appear to be directly infected by the rickettsia. Prevalence of the rickettsial microcolonies was 80% in shrimp from pond 1, 66.6% from pond 2 and absent in pond 3.

The absence of a detectable rickettsial infection in pond three coincided with the absence of cellular aggregation and granulomas in these shrimp, although small dense granulomas composed of 10 to 20 necrotic cells were present in the heart, peripheral hepatopancreas and, very occasionally, in the gill branches (Fig. 6). There was a clear artifactual space between the foci of compact necrotic cells and the surrounding mild host reaction, which consisted of one or two concentric layers of fibrocytes. This appeared to be a chronic resolution stage of an earlier inflammatory response.

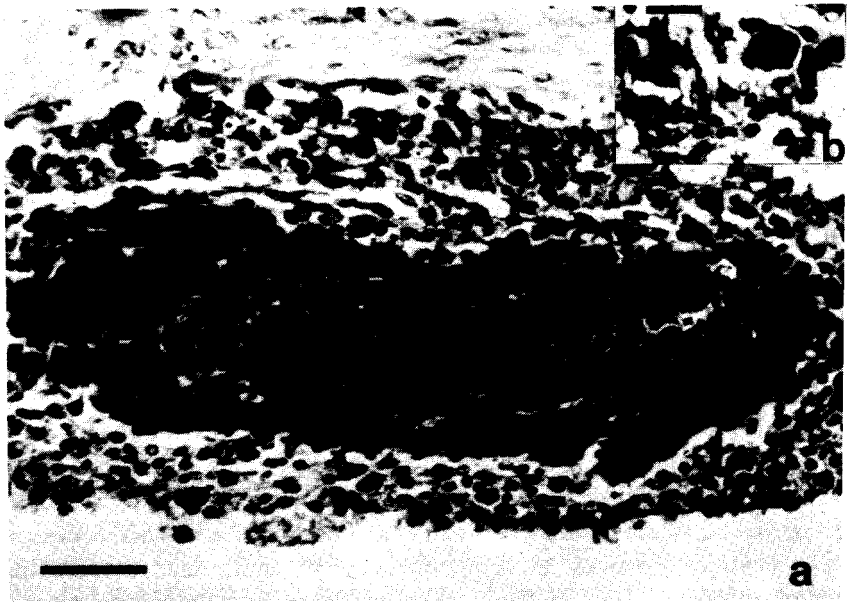

Fig. 6. a) A melanised granuloma. Note the caseous center, zone of compact melanised hemocytes and the loosely arranged outer zone of hemocytes, fibrocytes and rickettsial microcolony-containing cells. H & E; bar = 40 μm. b) Small dense granulomas in the heart. H & E; bar = 20 μm.

Gram-negative straight and curved rods were present in tissues of shrimp from all ponds, with an overall prevalence of 43%. Most commonly small colonies were observed within the heart, closely associated with the cardiac muscle fibers (Fig. 7a), gill filaments and at the periphery, or in the distal intertubular tissues of the hepatopancreas. There was little pathology associated with these focal colonies, with only some slight degenerative changes in cells close by. An absence of epithelium and sloughing with growth of Gram-negative bacteria in the midgut lumen and on the lining was observed in a shrimp from pond 1 and another from pond 2. Occasionally an entire proximal tubule hepatopancreatocytic lining would be absent and the remaining tissue space entirely filled with bacteria. Several shrimp from ponds 1 and 2 had a generalized bacteremia. The gill filaments would be markedly dilated and the filament epithelial cells necrotic (Fig. 7b). There was a diffuse degeneration of the hepatopancreas with an unusual fragmentation of nucleoli into many small basophilic droplets, a necrosis and sloughing

Fig. 7. a) Gram-negative bacterial colonies (arrowheads) and rickettsial microcolonies (arrows) within the heart. Gram's stain; bar = 40 μm. b) Dilated gill filaments seen in generalized bacterial septicemias. Note the melanised granuloma in the gill branch and gill fouling. H & E; bar = 100 μm.

of hepatopancreatocytes and focal areas of muscle necrosis. Bacteria were obvious in any tissue space where the hemolymph circulated.

Gill fouling, most marked in shrimp with bacteremias, was present to some degree in the majority of gill sections examined (Fig. 7b). The fouling consisted mostly of finely granular material, but the occasional *Epistylis*-like protozoan and algae were also apparent. A reactive hyperplasia, with mitotic figures of hematopoietic cells, was apparent histologically, though rickettsial microcolonies were entirely absent in all hematopoietic tissues.

In shrimp from ponds 1 and 2, the pathology associated with MBV, rickettsial and bacterial infections, was observed in the same animal. In all cases, it was the rickettsia-associated cell aggregations and granulomas that were most marked. In those cases of severe systemic bacteremias, bacteria contributed significantly to other pathological lesions. The occurrence and prevalence of the different pathogens in the shrimp examined histologically are given in Table 1. The specimens examined were too few to estimate a population prevalence.

Table 1. Prevalence of rickettsia, MBV and bacteria in *P. monodon* sections including hepatopancreas, examined histologically.

Pond	Number of Specimens	MBV Only	B Only	MBV & B	R & MBV	R & B & MBV	R & B
1	5	1	-	-	2	1	1
2	6	-	-	-	2	3	1
3	7	2	1	2	-	-	-

MBV = *Penaeus monodon* Baculovirus.
B = Gram-negative bacteria.
R = Rickettsia-like organisms.

Discussion

The etiology of the mortalities was not clear from clinical examination. Affected prawns exhibited nonspecific symptoms and gross signs similar to a variety of conditions (e.g., bacterial septicemias (Lightner and Lewis 1975); MBV infections (Lightner et al. 1983); and protozoan epicommensal gill fouling (Lightner 1977)) and/or as a result of a nonspecific depression of normal preening behavior, molting and feeding. The small, 0.5 mm white nodules, apparent in the midgut wall, have not previously been described and were the only gross lesions unique to this condition. Histopathological examination of the shrimp from affected ponds revealed a variety of lesions, some very severe, relating to different pathogens.

The changes associated with MBV and the tissue distribution of the virus were essentially identical to those described by Lightner et al. (1983). Additionally, degenerating stage 4 hepatopancreatocytes (distinct eosinophilic intranuclear occlusions present in the nuclei) possessing large clear cytoplasmic vacuoles were frequently noted. The presence of many normal uninfected hepatopancreatocytes (and hepatopancreatic tubules) suggests that the virus itself was insufficiently pathogenic to cause the observed mortalities. In their detailed study, Lightner et al. (1983) concluded that the lethality of MBV infection is increased by the presence of other pathogens. In this case, marked tissue destruction was related to the presence of bacteria and a rickettsia-like organism.

Although electron microscopy did not reveal a trilaminar cell wall, the intracytoplasmic, Gram-negative, rod-shaped organism appeared to belong to the family Rickettsiaceae (Moulder 1974). Rickettsial microcolonies present in affected shrimp were similar to those described in *P. marginatus* and *P. stylirostris* (Brock et al. 1986), but in this case, while the distribution of the rickettsia-infected cells in *P. monodon* was very wide, involving all connective tissues, the hepatopancreatocytes remained apparently unaffected. This was similar to *Carcinus mediterraneus,* another marine decapod crustacean, in which Bonami and Pappalardo (1980) found rickettsia in the cytoplasm of connective tissue cells of the hepatopancreas, gut, gills and gonads. In the only other recorded rickettsial infection of marine crustaceans, Johnson (1984a) reported the presence of rickettsial microcolonies in hepatopancreatocytes of *Paralithodes platypus.* In *P. monodon,* at the rickettsial microcolony tissue sites, there was often tissue necrosis and a marked host response, involving hemocyte aggregations and encapsulations with compact melanised hemocytes and fibrocytes. The reactive hyperplasia present in hematopoietic tissues may have reflected the hosts' response to depletion of circulating hemocytes following their aggregation at inflammatory foci. These inflammatory changes are a typical response of decapod crustaceans to an irritant (Sparks 1985), but have not been reported in the other decapod rickettsial infections (Bonami and Pappalardo 1980; Johnson 1984a; Brock et al. 1986). Rickettsial microcolonies and the associated hemocyte aggregations in the midgut wall appeared to produce the lesion that was grossly apparent as small white nodules. While the tissue distribution of rickettsia-associated lesions was wide, those tissues or organs in which a large volume of hemolymph passes, that is the heart, hepatopancreas, Y-organ, gills and parietal hemocoele linings

(Johnson 1980), showed the most notable lesions. The near total destruction of the Y-organ cannot be explained. Due to the extensive tissue destruction associated with the rickettsia, the organism was probably primarily responsible for the mortalities observed on the farm. However, in the majority of specimens with detectable rickettsial infections (8/12) there was an associated bacteremia.

The presence of bacteria in the diseased shrimp was not unexpected as systemic bacterial infections in penaeids can follow severe stress, cuticular injuries or weakening of defense mechanisms due to other diseases (Lightner 1977, 1983). The Gram-negative rods may be members of the genus *Vibrio*. Lightner (1983) states that most isolates reported from bacterial septicemias in penaeids have been *Vibrio* species. In bacterial septicemias investigated at other times we isolated bacteria of the same genus, most commonly *V. alginolyticus* (unpublished data). Discrete bacterial colonies were observed in the heart, gill filaments and hepatopancreas. In experimental *Vibrio* infections in *Callinectes sapidus* (Johnson 1976) and *Penaeus aztecus* (Lewis 1974) bacteria were concentrated in the same sites. This represents the early bacterial infection, but in several shrimp the infection had progressed to a point where bacteria were disseminated through all tissues containing hemolymph. Severe pathological changes in the gills (necrosis and filament swelling) and hepatopancreas (pyknotic necrosis of hepatopancreatocytes), and milder changes in the skeletal musculature (focal necrosis), occurred in the massive systemic forms of the bacterial infection. The proliferation of hindgut bacteria, apparent in two specimens, is likely to be due to the loss of homeostasis in the moribund state. Unlike the host response to Gram-negative infections in *Carcinus maenas* (Smith and Ratcliffe 1980) and *Callinectes sapidus* (Johnson 1976), the bacteria were never seen to be associated with hemocyte aggregations or the small dense granulomas. As the host response appeared to be primarily directed at the rickettsia, the bacteremia was considered to be the more recent infection, indicating that the bacterial infection was secondary to the rickettsial or viral infections. Even as secondary pathogens, the bacteria seem to have been a likely lethal terminating agent considering the severe pathology attributable to them in the extensive bacteremias.

Since experimental infections were not undertaken, no certainty can be attributed to a proposed primary etiology of the condition investigated. Possibly *Penaeus monodon* is an abnormal host for the rickettsia, similar to the rickettsia from *P. marginatus* in *P. stylirostris* (Brock et al. 1986). No rickettsial microcolonies or

intracytoplasmic rickettsia were detected in shrimp sampled from pond 3. Similarly, sampling from the remaining population in pond 2 three weeks later failed to detect any rickettsial infection, although a low prevalence of stage 3 MBV hepatopancreatic nuclei were present. It is possible that rickettsia were present but that normal histological methods did not allow detection (Johnson 1984a; Brock et al. 1986). Neither were any *P. merguiensis* or *P. indicus,* reported to be present in the pond and originating from the wild, examined histologically to determine whether they represented a carrier population for the rickettsia. Since rickettsia are obligate intracellular parasites, a carrier population of aquatic animals must exist. What they are and how the organism is transmitted are not known.

MBV is likely to be normally present in all cultured pond populations of *P. monodon* in Malaysia at low endemic levels, as wild-caught broodstock are used as spawners and the virus is enzootic throughout the region (Lightner 1985). The presence of the reo-like virus in the same population (Nash et al., in press) is an additional factor to consider, but one of unknown significance. The importance of environmental stressors in initiating the epizootic cannot be discounted since it has been reported that in infections of MBV, *Baculovirus penaei* and infectious hypodermal and hematopoietic necrosis virus (in *P. vannamei*) environmental stressors increase the prevalence of patent infections and disease (Couch et al. 1975; Lightner et al. 1983; Lightner 1985). In this case, because management and environmental manipulation had no effect on the incidence of mortalities, it is unlikely that environmental factors had a primary role. This conclusion was supported by observations at the farm that wild *P. merguiensis* and *P. indicus* grew well, even though *P. monodon* are considered to be more tolerant to environmental fluctuations and low salinity than these other species (Ong 1983). Occurrence of simultaneous viral infections in laboratory populations of marine crustaceans is not unknown (Johnson 1984b), but a multiple viral infection together with a rickettsial infection has not been reported. The pathogenesis of such multiple infections thus can only be speculated. The authors are inclined to believe that the rickettsia was the primary pathogen with secondary bacterial infections contributing to the mortalities seen. MBV and the reo-like virus may have become patent following weakening of the shrimp or may possibly facilitate, synergistically or additionally, the initial rickettsial infection.

Subsequently, production on the affected farm was changed to *P. merguiensis*. The production obtained with this species has been high

and there has been no reccurrence of mortalities. Due to the presence of wild shrimp in the supply channels and water intake areas (possible sources of the rickettsia), the high cost of antibiotic-medicated feeds which could be used to control rickettsial and secondary bacterial infections, and a good market price for *P. merguiensis*, the change of species was considered the most practical solution to the farm's problems. With the growing awareness that viruses, rickettsia and chlamydia are pathogens in marine crustaceans (Sparks 1985), aquatic animal pathologists must be aware that, even in extensive pond culture, diseases may be seen that have not yet been described let alone defined. Investigation of all pond mortalities should always be undertaken, if only to confirm there is no infectious etiology.

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