

Reo-like virus in white shrimp *Penaeus vannamei* (Crustacea: Decapoda): co-occurrence with *Baculovirus penaei* in experimental infections

Rena M. Krol, William E. Hawkins, Robin M. Overstreet

Gulf Coast Research Laboratory, PO Box 7000, Ocean Springs, Mississippi 39564, USA

ABSTRACT: A virus, tentatively identified as reo-like, occurred concurrently with experimentally-induced *Baculovirus penaei* (BP) infection in cultured white shrimp larvae *Penaeus vannamei*. Each shrimp with a reo-like viral infection also had a BP infection, but not all BP-infected shrimp had a reo-like infection. Both viruses occurred in the same tissues and occasionally within the same cell. The reo-like virus developed in epithelial cells of the anterior midgut and in reserve- and fibrillar-cells of the hepatopancreas. The paraspherical and non-enveloped reo-like virions (ca 50 nm diam.) occurred as unordered aggregates in the cell cytoplasm. Their etiology has not been determined. Reo-like virions may have been introduced along with the BP virus, or, were latent and only manifested due to stress induced by the more pathogenic BP virus.

INTRODUCTION

Reo-like viruses have been reported in several species of crabs and shrimp. The paralysis virus of the portunid crab *Macropipus depurator* (Vago 1966, Bonami 1973, Bonami et al. 1976, Bergoin et al. 1982) and the reo-like virus of the blue crab *Callinectes sapidus* (Johnson & Bodammer 1975, Johnson 1977, 1984), are associated with fatal paralysis of their hosts. In the Mediterranean shore crab *Carcinus mediterraneus*, a highly pathogenic, sometimes fatal, reo-like virus infecting the gill epithelium (Bonami 1977, Bergoin et al. 1982), and a reo-like virus, of undetermined pathogenicity, infecting the hepatopancreatic epithelium (Mari & Bonami 1987) have been reported. With regard to shrimp, reo-like virus appeared to have limited pathogenicity in the kuruma shrimp *Penaeus japonicus* (Tsing & Bonami 1987). Nash et al. (1988) recently reported a reo-like virus in dying giant tiger shrimp *P. monodon*, but could not determine whether the virus was the cause of death. Most of these reo-like viruses occur along with other viral infections, or are associated with other diseases in the same host.

During studies on the effects of *Baculovirus penaei* (BP) in larval white shrimp *Penaeus vannamei*, we observed a second virus tentatively identified as a reo-like virus in the midgut and hepatopancreatic

epithelium. This reo-like virus occurred only in specimens infected with BP. Here, we report on the ultra-structure and cytopathology of this virus and its relationship to BP.

MATERIAL AND METHODS

Naupli stage *Penaeus vannamei* were purchased from a commercial hatchery. Approximately 150 larvae were placed in each of ten 1 l Imhoff settling cones. When larval shrimp developed to the Protozoal III stage, larvae in 5 of the cones were infected with BP virus. Larvae in the other 5 cones served as controls; they were not exposed to viral material and were maintained in a separate, virus-free room.

Details of experimental infection protocols have been reported elsewhere (Overstreet et al. 1988). Briefly, rotifers *Branchionus plicatilis* were fed minced larval shrimp tissues with microscopically confirmed BP infections. The BP-infected tissues had been collected from previous bioassays and stored at -70°C . Tissues were thawed and pulverized into a paste. Rotifers were exposed to the BP-infected tissues for 2 to 3 h and then fed to shrimps. After protozoal shrimp were exposed to rotifers for 26 h, rotifers and uneaten debris were removed and the water replaced. Both experimentally

infected and negative control larvae were maintained in cones and fed a diet of brine shrimp *Artemia salina*.

Exposed and control larvae were examined by light and electron microscopy at 96, 117.5 and 142 h post shrimp-exposure. For electron microscopy, shrimp were cut into cephalothorax and tail portions and fixed in 2.0 % glutaraldehyde in 0.025 M sodium cacodylate buffered seawater. Specimens were postfixed in 1.0 % osmium tetroxide in 0.1 M sodium cacodylate buffer, dehydrated in a graded series of ethanols, and embedded in Spurr's resin (Spurr 1969). Specimens were oriented in flat molds so that cross-sections of cephalothorax could be examined for systemic viral infection. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963) and examined with a JEOL 100 SX electron microscope at 60 KV.

RESULTS

Twenty-three shrimp, 14 experimentally infected and 9 negative controls, from 96, 117.5 and 142 h samples were examined by light and electron microscopy to determine the co-occurrence of BP and reo-like infections. Nine of 14 shrimp fed BP-infected material showed morphological evidence of BP infection and 5

of the 9 also contained reo-like virus. None of the 9 controls examined exhibited evidence of either virus.

Polyhedral occlusion bodies characteristic of BP infection, as well as free BP virions, were present in nuclei of epithelial cells of the hepatopancreas and anterior midgut of experimentally infected shrimp. The reo-like virus occurred as small clusters of virions in the cytoplasm of hepatopancreatic and anterior midgut epithelial cells (Fig. 1). Occasionally, both viruses occurred in the same cell (Fig. 2). The reo-like virus most frequently infected reserve (R) cells and, to a lesser extent, fibrillar (F) cells of the hepatopancreas (Fig. 3). The virus occurred as unordered aggregates of virions in a filamentous matrix which resembled a virogenic stroma. The virions were 40 to 60 nm in diameter, paraspherical, and non-enveloped (Fig. 4). The outer shell of the virion appeared fragile and often lost its structural integrity.

DISCUSSION

Based on shape, size, and cytoplasmic location, the virus described here belongs to the family Reoviridae. Positive identification, however, depends on isolation, purification and analysis of its nucleic acid. The reo-

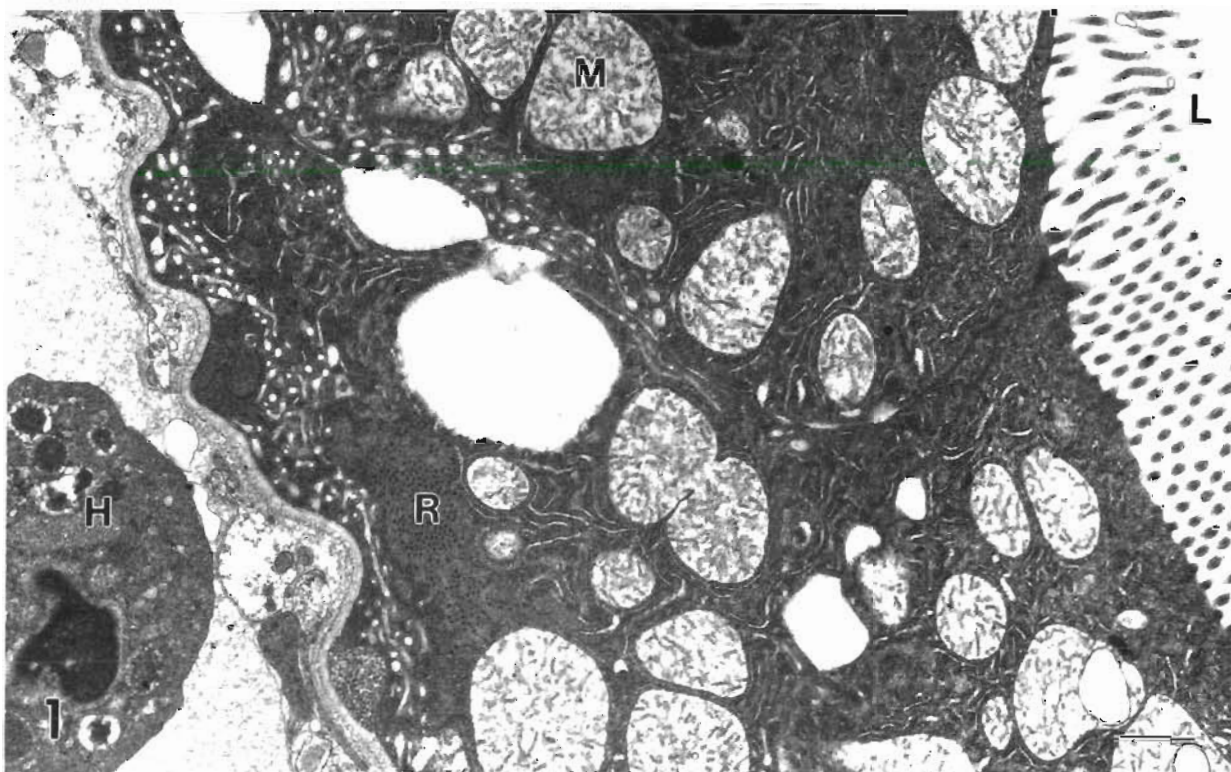
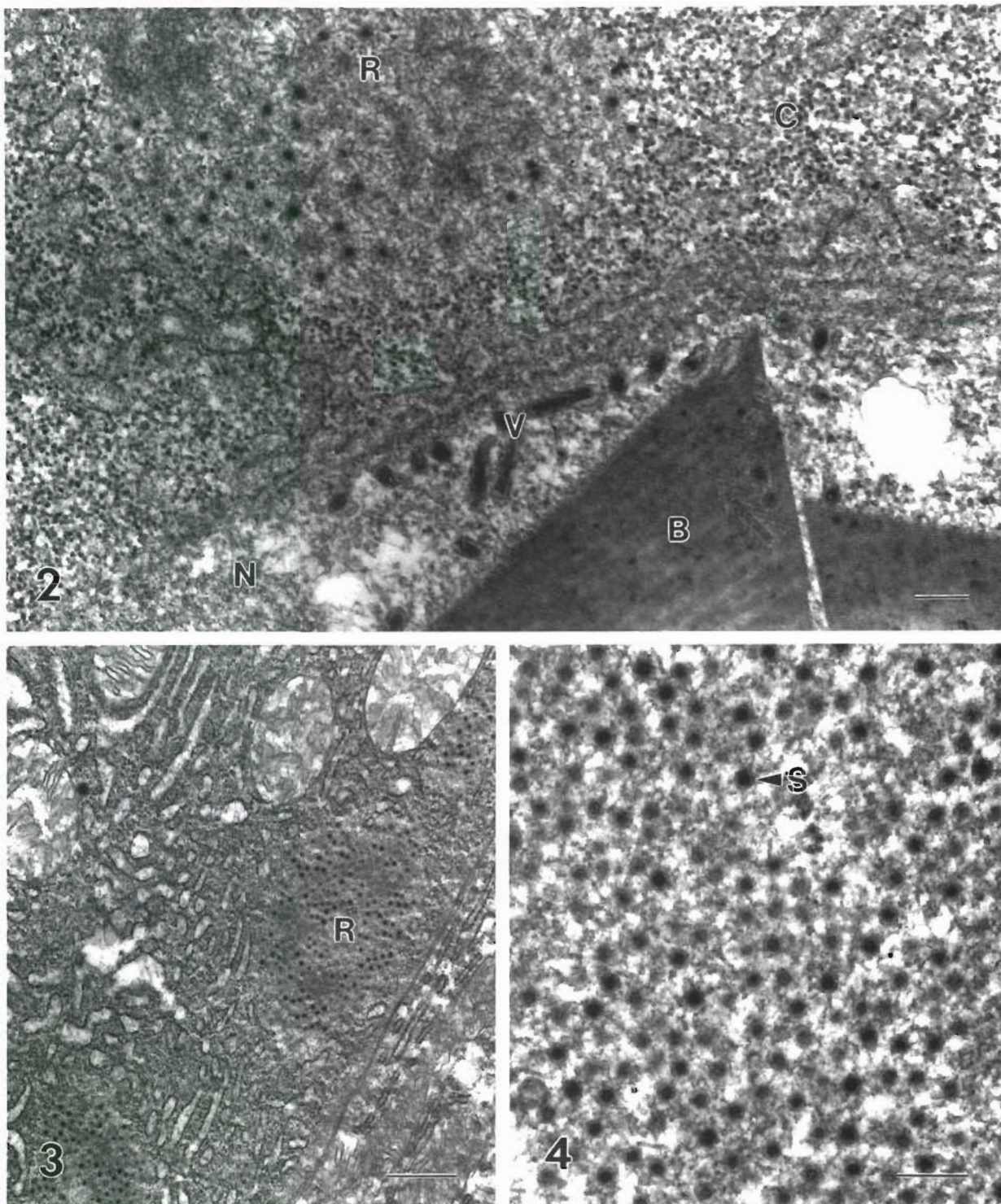


Fig. 1. *Penaeus vannamei*. Reo-like virus infection of anterior midgut epithelium. R: reo-like virions; M: mitochondria; L: midgut tubule lumen; H: hemocyte. Bar = 1 μ m



Figs. 2 to 4. *Penaeus vannamei*. Fig. 2. Concurrent BP and reo-like infections in hepatopancreas cell. R. reo-like virions, B BP polyhedron with sequestered virions; V. free BP virions; N nucleus. C cytoplasm. Bar = 200 nm Fig. 3. Reo-like infection in F-cell of hepatopancreas; R. reo-like virions. Bar = 500 nm Fig. 4. Reo-like virions; S. outer shell. Bar = 200 nm

like virus in *Penaeus vannamei* not only infected hepatopancreatic cells as did the reo-like viruses described in *P. japonicus* by Tsing & Bonami (1987) and in *P. monodon* by Nash et al. (1988), but also epithelial cells of the anterior midgut. Virions in both *P. vannamei* and in *P. japonicus* occurred in unordered aggregates primarily in R- and F-cells (Sindermann & Lightner 1988) in contrast with virions in *P. monodon* which sometimes occurred in paracrystalline arrays. The 40 to 60 nm diameter virions of *P. vannamei* were somewhat smaller than those in *P. monodon* (50 to 70 nm diam.) and in *P. japonicus* (61 nm diam.).

Several reo-like viruses infect crabs but only the RC84 virus of *Carcinus mediterraneus* resembles the reo-like viruses reported in shrimp (Mari & Bonami 1987). The RC84 virus is restricted to the hepatopancreas, primarily in blister (B) cells, but also in R-cells in heavy infections. Like the reo-like virus in *Penaeus vannamei*, the RC84 virions are usually found in an unordered arrangement.

Examples of simultaneous viral infections with 2 or more viruses infecting the same organism have been reported in several crustaceans. An unidentified virus was found in a purified preparation of reo-like virus from *Penaeus japonicus* (Tsing & Bonami 1987). Reo-like viruses were associated with *P. monodon* baculovirus (Nash et al. 1988), with a bunya-like virus in *Macropipus depurator* (S-P syndrome) (Bonami 1973), with a picorna-like virus in the parasitic isopod *Portunio conformis* (Kuris et al. 1979), and with a virus in connective tissue of *Carcinus mediterraneus* (Mari & Bonami 1987). Johnson (1984) considered rhabdo- and reo-like infections in the blue crab *Callinectes sapidus* to be synergistically pathogenic. That does not appear to be the case, however, with BP and the reo-like virus described in this report.

Although multiple viral infections of various tissues often occur in individual crabs, the co-occurrence of viral infections in digestive epithelium has not been reported (Mari & Bonami 1987). This contrasts with the BP and reo-like infections in *Penaeus vannamei* which develop not only in the digestive epithelium of the same individual but occasionally in the same cell.

We cannot determine from this study whether the reo-like virus in *Penaeus vannamei* was introduced with *Baculovirus penaei* (BP) in experimental infections, or whether the reo-like virus was latent and appeared secondarily to stress brought on by the highly pathogenic BP. The presence of reo-like viruses in several other crustaceans, however, seems certainly to be related to stress, since the viruses usually occurred in organisms sensitive to other pathogens. Tsing & Bonami (1987) suggested that the reo-like virus in *P. japonicus* may be a predisposing stress-factor leading to other disease conditions. For example, shrimp with

reo-like infections were often infected with the imperfect fungus *Fusarium* sp. Because reo-like virus infections in *P. japonicus* often occurred in shrimp that otherwise appeared healthy, the virus probably was not pathogenic (Sindermann & Lightner 1988). An experimental reo-like infection in *P. japonicus* developed slowly, with no mortalities occurring until 45 d post infection (p.i.) (Tsing & Bonami 1987). The light infections of reo-like virus that we observed at 96 to 142 h p.i. were concomitant with heavy infections of BP. Because BP is highly lethal to larval shrimp, with almost 100 % loss of the population within 1 to 2 d of the appearance of infection (Overstreet et al. 1988, Sindermann & Lightner 1988), it is likely that the reo-like virus also exhibits a limited pathogenicity in *P. vannamei* because of its apparently slow developmental time.

Acknowledgements. We thank Mary Ann Vogelbein for her technical assistance and Dr Donald Lightner, University of Arizona, for his valuable comments on this study. This study was supported in part by U.S. Department of Agriculture, Cooperative State Research Service, Grant No. 88-38808-3319.

LITERATURE CITED

- Bergoin, M., Bonami, J.-R., Morel, G. (1982). Crab and spider viruses. Proc. int. Colloq. Invertebr. Path. 3: 523-526
- Bonami, J.-R. (1973). Recherche sur la paralysie virale du Crustacé Décapode *Macropipus depurator* L. Revue Trav. Inst. (scient. tech.) Pêch. marit. 37: 387-389
- Bonami, J.-R. (1977). Les maladies virales des crustacés et des mollusques. Océanis, Paris 3: 154-175
- Bonami, J.-R., Comps, M., Veyrunes, J.-C. (1976). Etude histopathologique et ultrastructurale de la paralysie virale du crabe *Macropipus depurator* L. Revue Trav. Inst. (scient. tech.) Pêch. marit. 40: 139-146
- Johnson, P. T. (1977). A viral disease of the blue crab, *Callinectes sapidus*: histopathology and differential diagnosis. J. Invertebr. Path. 29: 201-209
- Johnson, P. T. (1984). Viral diseases of marine invertebrates. Helgoländer Meeresunters. 37: 65-98
- Johnson, P. T., Bodammer, J. E. (1975). A disease of the blue crab, *Callinectes sapidus*, of possible viral etiology. J. Invertebr. Path. 26: 141-143
- Kuris, A. M., Poinar, G. O. Jr, Hess, R., Morris, T. J. (1979). Virus particles in an internal parasite, *Portunio conformis* (Crustacea: Isopoda: Entonsicidae), and its marine crab host, *Hemigrapsus oregonensis*. J. Invertebr. Path. 34: 26-31
- Mari, J., Bonami, J.-R. (1987). A reolike virus of the Mediterranean shore crab *Carcinus mediterraneus*. Dis. aquat. Org. 3: 107-112
- Nash, M., Nash, G., Anderson, I. G., Shariff, M. (1988). A reo-like virus observed in the tiger prawn, *Penaeus monodon* Fabricius, from Malaysia. J. Fish Dis. 11: 531-535
- Overstreet, R. M., Stuck, K. C., Krol, R. A., Hawkins, W. E. (1988). Experimental infections with *Baculovirus penaei* in the white shrimp *Penaeus vannamei* (Crustacea: Decapoda) as a bioassay. J. Wild Aquaculture Soc. 19: 175-187

- Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208–212
- Sindermann, C. J., Lightner, D. V. (eds.) (1988). Disease diagnosis and control in North American marine aquaculture. *Developments in Aquaculture and Fisheries Science*, Vol. 17, 2nd edn. Elsevier Science Publishers, The Netherlands
- Spurr, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26: 31–43
- Tsing, A., Bonami, J.-R. (1987). A new viral disease of the tiger shrimp, *Penaeus japonicus* Bate. *J. Fish Dis.* 10: 139–141
- Vago, C. (1966). A virus disease in Crustacea. *Nature, Lond.* 209: 1290

Responsible Subject Editor: Dr J. E. Stewart, Dartmouth, N.S., Canada

Manuscript first received: July 4, 1989
Revised version accepted: December 1, 1989