Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by yellow-head disease

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ABSTRACT: A recently reported disease syndrome of *Penaeus monodon* in Thailand is called 'yellow-head' or 'hua leung' in Thai. It is usually characterized by light yellow coloration of the dorsal cephalothorax area and generally pale or bleached appearance of affected prawns. The yellow color in the cephalothorax region results from the underlying yellow hepatopancreas showing through the translucent carapace in moribund shrimp. In histological preparations of moribund yellow-head specimens for the light microscope, no consistent bacterial, fungal or parasitic agents could be found. The lymphoid organs of yellow-head specimens showed extensive abnormalities. These included obviously necrotic cells and vacuolated cells with hypertrophied nuclei. Also evident were very densely basophilic, globose cytoplasmic inclusions located adjacent to some of the hypertrophied nuclei. Similar basophilic inclusions were found in interstitial hepatopancreatic tissue, in connective tissue underlying the mid gut, in cardiac tissue, in gill tissue and in hematopoietic tissue. Transmission electron micrographs revealed the presence of previously undescribed rod-shaped, enveloped virions in the cytoplasm adjacent to the nuclei of cells from various tissues. Free virions were also present in intercellular spaces. The virions were similar to those of the insect granulosis viruses (Baculoviridae) in terms of cytoplasmic location, size, morphology and development. However, they were not occluded by granulin.

KEY WORDS: Baculovirus · *Penaeus monodon* · Thailand · YBV · Yellowhead disease

INTRODUCTION

In 1990, Limsuwan (1991) first noted the occurrence of 'yellow-head' ('hua leung' in Thai) as a new disease syndrome in pond-reared black tiger prawns *Penaeus monodon* in central Thailand. He named this syndrome based on the light yellow coloration of the dorsal cephalothorax area and the generally pale or bleached appearance of affected shrimp. The yellow color in the cephalothorax region resulted from the underlying yellow hepatopancreas showing through the translucent carapace in moribund shrimp.

According to Limsuwan (1991), this syndrome occurs in pond-reared shrimp 5 to 15 g in size. He described the development of the syndrome as follows. At first, the shrimp begin by consuming feed at an abnormally high rate for several days. Then they cease eating entirely and within 1 d of cessation, a few moribund shrimp appear swimming slowly near the surface at the edge of the pond. These shrimp have a light yellow cephalothorax as described above and die within a few hours. By the following day, the number of similarly affected shrimp increases dramatically, and by the third day after the first appearance of moribund shrimp, the entire crop is lost. Dead shrimp are found at the edge of the pond and scattered evenly over the entire

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bottom of the pond. By contrast, coexistent banana prawns *Penaeus merguensis* previously acquired incidentally as larvae in the inlet water remain healthy and without signs of disease. This apparent specificity led Limsuwan (pers. comm.) to suggest that a viral agent might be implicated.

Histological examination of the shrimp (Limsuwan 1991) revealed abnormally high quantities of fat in hepatopancreatic tubule cells of all affected shrimp, suggesting that the normal pathways of fat utilization had been interrupted. Some of the affected prawns had systemic bacterial infections or infections of monodon baculovirus (MBV), but these features were not consistent or particularly severe and are known to be found commonly in non-yellow-head specimens. No other infectious agents were seen. Examination of the water from affected ponds (Limsuwan 1991) revealed relatively high levels of organophosphate insecticides, indicating that they might be associated with the syndrome.

'Yellow-head' syndrome was first recognized in 1990 in central Thailand in the shrimp farming areas near Bangkok (i.e. Samut Sakorn and Samut Prakan). It was subsequently reported from more southerly farming areas on both sides of the Gulf of Thailand (down to Rayong on the east coast and down to Prachuap Kirikhan on the west) (Limsuwan 1991). In the far south it was found first in February 1992 (Flegel et al. 1992) and has since become more widely spread in that region. This report presents observations of pond outbreaks of yellow-head disease and results of histological examination with light and electron microscopes of specimens collected from these ponds in southern Thailand between February and August 1992. It also includes data from a re-examination of normal broodstock specimens for the presence of the same virus.

MATERIALS AND METHODS

Source and preparation of specimens. More than 1000 prawns *Penaeus monodon* collected from approximately 50 ponds between February 1992 and August 1992 were used in this study. Of the ponds sampled, all experienced total mortalities within 3 to 5 d, except where the ponds had been emergency harvested beforehand. Shrimp from all but one of these high mortality ponds showed typical gross clinical signs of yellow-head disease.

Captured broodstock shrimp were prepared for histological examination at the end of their useful hatchery phase (duration approximately 1 mo). These were normal specimens without any gross signs of disease.

Preparation of specimens for normal histology was done according to Bell & Lightner (1988) while prepar-

ation of specimens for the electron microscope was as described by Fegan et al. (1991).

Bacterial isolations. Approximately 300 yellow-head specimens were screened for systemic bacterial infections by plating of hemolymph on tryptic soy agar supplemented with 2 % sodium chloride (marine TSA). The telson of live shrimp was swabbed with 70 % ethanol and then cut with a pair of flame sterilized scissors. The exuded hemolymph was dripped onto the agar surface and subsequently streaked. Plates were incubated at 30 °C overnight and inspected for presence of bacterial colonies. Any colonies arising were sampled by subculture on TCBS agar supplemented with 2 % sodium chloride (marine TCBS). Those giving good growth on TCBS were labeled as presumptive *Vibrio* isolates.

RESULTS

Indicative gross signs of yellow-head shrimp

More than 50 ponds with yellow-head syndrome were visited between February 1992 and November 1992. The clinical signs of the moribund shrimp in these ponds were usually similar to those described by Limsuwan (1991) except that a distinct yellowing of the gills was also noted. The course of the disease was the same, in that accumulative mortalities reached up to 100 % in 3 to 5 d unless ponds had been emergency harvested. That is the current advice to farmers; to harvest ponds at the first sign of disease onset. This can help to reduce, but not eliminate, the economic loss which can still be considerable because of a premature harvest.

In addition to the ponds with clear gross signs of yellow-head, one other pond was visited where a rapid mortality pattern similar to that for yellow-head was seen but where the shrimp did not have a yellow cephalothorax. Histological examination at the light microscope level revealed the presence of basophilic, cytoplasmic inclusions similar to those found in yellow-head specimens, but no electron microscope studies were done to confirm the presence or absence of virus particles in these specimens (see 'Histology' below).

Bacterial isolations

Only 10 % of the moribund, yellow-head shrimp examined gave positive isolations for systemic bacteria. All exhibited good growth on marine TCBS agar and were labeled as presumptive strains of *Vibrio*, a common opportunistic pathogen.

Light microscope histology

Examinations were confined to moribund yellow-head shrimp specimens without systemic bacterial infections, and to normal captured broodstock. With the light microscope, the yellow-head specimens stained with hematoxylin and eosin revealed nothing abnormal in the cells of the hepatopancreatic tubule epithelium, except for large numbers of fat droplets in the R and B cells. There was no evidence of bacterial, fungal or parasitic agents. Many prawns did show typical inclusion bodies of monodon baculovirus (MBV) and very few were infected with hepatopancreatic parvovirus (HPV). However, these 2 viruses have also been found in shrimp specimens not affected by yellow-head syndrome.

In contrast to the hepatopancreatic tubule cells, the lymphoid organs of yellow-head specimens showed extensive abnormalities. These included obviously necrotic cells and cells with hypertrophied nuclei and large vacuoles (Figs. 1a, b & 2a). These cells tended to

appear in areas resembling degenerate tubules with occluded lumens. The abnormal tissue stained more basophilic than normal tubule tissue, but this difference was not always sharp, especially in early infection stages. The contrast was very clear with Feulgen staining, where the counterstain gave dark green with normal tubules in contrast to light green for abnormal tubules. Also evident in the H&E preparations were very densely basophilic, globose bodies similar to pycnotic nuclei (Figs. 1a, b & 2a). Examination of these densely basophilic globose bodies at high magnification revealed that many were not pycnotic nuclei but rather cytoplasmic inclusions located adjacent to nuclei. Some of the cytoplasmic inclusions were Feulgen positive, indicating possible DNA content (Fig. 2b).

Similar densely basophilic inclusions adjacent to nuclei were also found in various other tissues including interstitial tissues of the hepatopancreas (Fig. 2c), especially adjacent to the distal ends of the tubules, in the connective tissue underlying the mid gut, in cardiac tissue, in hematopoietic tissue (Flegel et al. 1992)

Fig. 1. *Penaeus monodon*. Histology of the lymphoid organ of yellow-head infected shrimp with H&E staining. (a) Early stage of infection showing 1 normal tubule at the top with the lumen marked (L). Surrounding tubules are occluded, the nuclei are hypertrophied and the cells are highly vacuolated. Also present (arrows) are densely staining basophilic inclusions. Scale bar = 15 µm. (b) Late stage of infection showing increased vacuolation and extensive necrosis. The tubule at the bottom with the lumen marked (L) shows considerable cellular necrosis when compared to the normal tubule in (a). Scale bar = 15 µm

Fig. 2. *Penaeus monodon*. Histology of yellow-head specimens with the light microscope. (a) High magnification (H&E) of the lymphoid organ showing densely basophilic inclusions (arrows). Scale bar = 6 μ m. (b) Feulgen staining of the same tissue showing Feulgen positive inclusions. Scale bar = 6 μ m. (c) Densely basophilic inclusions (H&E) in the interstitial tissue underlying hepatopancreatic tubule epithelial cells (arrows). Scale bar = 15 μ m. (d) Section of hepatopancreatic tissue from a suspected yellow-head specimen without a yellow cephalothorax. In a single field, there are cells infected with hepatopancreatic parvo-like virus (H), monodon baculovirus (M) and probably yellow-head virus (Y). Scale bar = 60 μ m.

and in gill tissue (Fig. 3a & b). In the H&E preparations, it was difficult to distinguish these densely staining bodies from pycnotic nuclei, except in sections where the inclusion and its adjacent nucleus were side by side. Indeed, some of the densely staining bodies were undoubtedly pycnotic nuclei, as was confirmed by examination with the electron microscope (see next section).

Specimens from 1 pond with rapid development of mortality, similar to that in yellow-head ponds, lacked a yellow cephalothorax but had abnormal lymphoid organs (LO) with basophilic cytoplasmic inclusions similar to those in yellow-head specimens. Some of these shrimp were also infected with both MBV and HPV (Fig. 2d). No follow-up on the electron microscope was carried out with these specimens so the presence or absence of the virus associated with yellow-head syndrome could not be confirmed. However, these observations suggest that infected shrimp might not display a yellow cephalothorax under all circumstances.

Electron microscope histology

From yellow-head tissues of the hepatopancreatic interstitial cells and the lymphoid organ, sections of Toluidine blue-stained material prepared prior to transmission electron microscopy revealed darkly staining cytoplasmic inclusions in some of the cells. These probably corresponded to the basophilic inclusions in the H&E preparations (Fig. 3c, d). With the transmission electron microscope, these basophilic inclusions consisted of 2 types of globose electron-dense bodies (Fig. 4a to c) and some pycnotic nuclei (Fig. 7d). Some were clearly composed of virogenic material (Fig. 4a, b, d, e). In the cytoplasm of many cells, there were long filaments (some over 800 nm in length) that appeared to be precursors of enveloped virions (Fig. 5a, b). These were sometimes densely packed, resembling paracrystalline arrays (Fig. 7d). The rod-shaped virions measured 173 ± 13 by 44 ± 6 nm ($n = 20$) on average and were often packed densely into vesicles, also resembling paracrystalline arrays (Fig. 7d). Free virions were also present in intercellular spaces probably by release from disintegrating cells (Fig. 5b).

Similar rod-shaped virions and filamentous precursors were found in normal broodstock specimens. In this material, the stages in viral development were followed and found to be similar to those in the yellow-head specimens. Specifically, rod-shaped elements (approximately 15 nm in diameter) appeared to issue from the nucleus and accumulate in the cytoplasm (Fig. 6 a,b). The filaments appeared to be in 2 forms, one more electron dense than the other. This may have represented material with and without cap-

sids (Figs. 4d, e, 5a & 6a, b). Viral envelopes appeared to be acquired by passage of the provirions through endoplasmic reticulum of the host cells in such a way that the virions collected together in vesicles (Figs. 6c & 7). The size of these vesicles, the number of enclosed virions and the density of packing varied widely (Figs. 6 & 7). Acquisition of capsids and envelopes often occurred while the viral material was still in the form of long filaments. Once enveloped, the filaments subsequently underwent fragmentation to produce the shorter, rod-shaped, enveloped virions (Figs. 5b, 6c & 7a). The viral envelope appeared somewhat fuzzy by the transmission electron microscope, possibly indicating the accumulation of some protein (granulin?) on its outer surface. In some cases this was reasonably thick (Figs. 7b, c).

DISCUSSION

General

Yellow-head syndrome is a newly reported disease of the black tiger prawn and this study has shown that affected shrimp contain a previously unknown cytoplasmic virus. The coincidence of a newly reported disease and a newly reported virus suggests that the virus is the causative agent of the disease. It is true that other viruses (i.e. MBV and HPV) were present in some of the shrimp examined. However, they were not ubiquitous in these animals and they are already known to occur in *Penaeus monodon* specimens which do not show the gross signs and rapid mortality described for yellow-head syndrome.

Disease development and gross signs

Based on the site observations in this study, the description of the course of yellow-head disease in rearing ponds must be modified. We concur with Limsuwan (1991) that appearance of clinical signs and mortality are always preceded immediately by complete cessation in feeding. However, we have found that cessation in feeding is not always preceded by a period of abnormally high feed consumption, as he reported. With respect to coloration, the characteristic of distinctly yellowish gills should be added to the appearance of a yellow cephalothorax. Finally, there is the precaution that we have visited a few ponds showing very high and rapid mortality similar to that seen with yellow-head but found no specimens with a yellow cephalothorax. This was in spite of the fact that the histological picture was similar to that for yellow-head specimens.

Fig. 3. *Penaeus monodon*. Histology of yellow-head specimens with the light microscope. (a) Low magnification of gill tissue (H&E) showing densely basophilic inclusions distinct from the nuclei. Scale bar = 15 μ m. (b) As in Fig. (a) except at higher magnification. Scale bar = 6 μ m. (c) Toluidine blue stained sections of hepatopancreatic interstitial tissue showing densely staining inclusions (arrows) that probably correspond to the densely staining basophilic material seen in the H&E preparations. Scale bar = 15 μ m. (d) As in Fig. (c) except at higher magnification. Scale bar = 6 μ m

Fig. 4. *Penaeus monodon*. Transmission electron microscopy of hepatopancreatic interstitial cells from yellow-head specimens. (a) to (c) Cytoplasmic inclusions of virogenic material adjacent to nuclei (arrows). Judging from the position and shape, these inclusions correspond to some of the densely staining inclusions seen in the H&E and toluidine blue stained specimens. Scale bars: (a) 1 μ m; (b) 2 μ m; (c) 2 μ m. (d) to (e) Progressively higher magnifications of the inclusion from (b) showing filamentous viral material scattered in the cytoplasm and associated with the electron-dense inclusion. The filaments associated with the inclusion appear more electron dense than those in the cytoplasm, suggesting that the inclusion body may consist of capsid material. Scale bars: (d) 500 nm; (e) 200 nm

Fig. 5. *Penaeus monodon*. Transmission electron micrographs of hepatopancreatic interstitial cells from yellow-head specimens. (a) A viral infected cell showing the nucleus (N), mitochondria (M), rough endoplasmic reticulum and large quantities of unenveloped viral filaments in the cytoplasm. The viral material is sectioned transversely and longitudinally. It appears in 2 densities, probably indicating the presence (dense) or absence (less dense) of capsid material. Scale bar = 200 nm. (b) Unenveloped virions (U) and enveloped virions (E) in the interstitial space, probably released from a ruptured cell. The average length of the short virions was approximately 170 nm. Note the double length virion (arrow). Scale bar = 200 nm

Fig. 6. *Penaeus monodon*. Transmission electron micrographs of viral development in the lymphoid organ of an asymptomatic broodstock specimen. (a) to (b) Low and high magnification of viral material (DNA cores?) issuing from the nucleus (N). Some rod-like structures are faintly visible in the nucleus in (b). Scale bars: (a) 300 nm; (b) 100 nm. (c) High magnification of filamentous viral material acquiring envelopes by passage through the endoplasmic reticulum. The process results in their accumulation in vesicles where they appear to divide into smaller units. Scale bar = 100 nm

Fig. 7. *Penaeus monodon*. Transmission electron micrographs of viral development in the lymphoid organ of an asymptomatic broodstock specimen and in hepatopancreatic interstitial cells of a yellow-head specimen. (a) High magnification of broodstock material showing virions accumulating in a vesicle. Scale bar = 100 nm. (b) Medium high magnification from a yellow-head specimen showing a cross section of a single enveloped virion surrounded by electron dense material and enclosed in a membrane. Scale bar = 200 nm. (c) High magnification of the lymphoid organ from an asymptomatic broodstock individual showing 7 enveloped virions each surrounded by a relatively thick layer of electron-dense material (granulin-like?) and all enclosed in a vesicular membrane. Scale bar = 100 nm. (d) Low magnification of hepatopancreatic interstitial cells of a yellow-head specimen showing paracrystalline arrays of unenveloped virions (U) and enveloped virions (E) adjacent to a pycnotic nucleus (N). Scale bar = 400 nm

Histology by light microscope

In contrast to obvious gross clinical signs, we have, as yet, found no clear way to characterize yellow-head virus infections in terms of standard histological examination with H&E-stained preparations. The problem is that cytoplasmic, virus-associated inclusions stain deeply basophilic in the same manner as pycnotic nuclei, and it is difficult to tell the two apart without the aid of the electron microscope. It is true that the LO is clearly abnormal in all the yellow-head infected individuals, showing nuclear abnormalities, cytoplasmic abnormalities and necrotic cells. However, these characteristics are also shared in whole or in part by penaeid shrimp infected with clearly different viruses (Owens et al. 1991, Bonami et al. 1992, Flegel et al. 1992). Feulgen staining might assist somewhat in helping to distinguish broadly between DNA and RNA viruses but the electron microscope would be required to make a final diagnosis. It may be that this LO morphology is characteristic of the general host response to viral or other infections and not characteristic of any particular virus. In fact, there is little published information on the function of the penaeid LO, or even on its normal histological characterization with the light and the electron microscope (e.g. only 4 references are cited in Bell & Lightner 1988). Detailed investigations on its response to viral or other infections would be useful.

Histology by electron microscope

The rod shaped, enveloped virions seen in the yellow-head specimens are similar to those of the insect granulosis viruses in terms of cytoplasmic location, size, morphology and development (Tanada & Hess 1991). As with some insect granulosis viruses, replication appeared to occur in the cytoplasm without any sign of virogenesis in the intact nuclei of infected cells. There was no evidence in our material of viral stromata or viral assembly in the nuclei and no evidence of nuclear membrane fragmentation to release virions into the cytoplasm. Rather, it appeared that the nucleic acid cores issued from intact nuclei and picked up capsids and envelopes in the cytoplasm. Because of the similarities to insect granulosis viruses (Baculoviridae), we propose that this agent is a new crustacean cytoplasmic granulosis virus and that it be called yellow-head baculovirus or YBV. This contention is supported by positive Feulgen staining of the cytoplasmic inclusions. Since the virus was widely distributed in the shrimp tissues examined, J. A. Brock (pers. comm.) has suggested that it be referred to as a systemic cytoplasmic granulosis virus. However, to confirm placement of this virus in the Baculoviridae, we must determine whether

the nucleic acid core consists of double stranded DNA and we should also have more detailed structural information derived from negatively stained EM preparations.

Although YBV bears some ultrastructural resemblance to insect cytoplasmic granulosis viruses, it also shows some differences. Most distinctive is the general lack of thick, clearly defined occlusion bodies or capsules of granulin enclosing the mature, enveloped virions (Tanada & Hess 1991). Also, there have been no reports for granulosis viruses of 2 very distinctive features of our material: paracrystalline-like arrays of nucleic acid cores and nucleocapsids, and the generation of mature virions by a process of fragmentation of rather long, somewhat flexible, enveloped filaments. Even so, these unique features would not be sufficient to exclude the virus from the Baculoviridae, should it be shown to contain double stranded DNA.*

Nature and source of yellow-head virus

We do not yet know whether this viral infection is a new one or a newly recognized one. Nor do we know its source or mode of transfer to pond-reared shrimp. However, in an earlier study of polyhedral viral-like material in LOs of normal, healthy, captured broodstock (Flegel et al. 1992), one specimen examined with the electron microscope showed the presence of cytoplasmic, enveloped, rod-shaped viral particles identical to those described here. This indicates that YBV may occur as latent, asymptomatic infections in broodstock shrimp and so there is a possibility of transfer from these shrimp to their offspring in larval rearing facilities. In addition, Lightner et al. (1987) reported a high incidence of LO abnormality in shrimp specimens collected during the dramatic decline in Taiwanese production of *Penaeus monodon* in the mid 1980s, although no associated viral agent was described. Subsequently, Chen & Kou (1989) reported the cultivation of MBV in LO tissue cultures, but the virions observed had dimensions of $58.4 \pm 10.2 \text{ nm} \times 165.9 \pm 29.3 \text{ nm}$. This is much smaller than MBV ($70 \times 360 \text{ nm}$; Fegan et al. 1991) but within the range of YBV reported here. Also in Chen & Kou (1989), the mature baculovirus virions in the tissue culture cells were found in the cytoplasm without occlusion bodies and they accumulated in vesicles. These features also resemble YBV rather than MBV. Thus, it may be that YBV was involved in the Taiwanese experience.

* While the manuscript was being processed, preliminary work by Jiraporn Kasornchandra showed that the nucleic acid from purified virions consisted of double stranded DNA (unpubl.)

In preliminary experiments (Boonyaratpalin et al. 1993a, b) carried out by some of our group at the National Institute of Coastal Aquaculture (NICA), yellow-head disease has been serially transferred over 7 times by injection of membrane-filtered (0.22 µm pore size), hepatopancreatic extracts at dilutions up to 10^7 , starting with an original pond-infected individual. The experimentally infected prawns developed identical clinical signs to the naturally infected prawns (faded color, light yellow gills and yellow HP) within 24 h, and cumulative mortality was 100 % within 3 d. These tests at NICA also indicated that other shrimp species could be artificially infected by injection (e.g. *Penaeus merguensis* and *Metapenaeus ensis*) and that extracts of krill *Acetes* sp. from yellow-head infected ponds caused the disease when injected into *P. monodon* in the laboratory.

All the preceding evidence suggests that yellow-head is not a new disease in Thailand but simply one which has been recently recognized, probably because of a dramatic increase in occurrence and severity. There are several possible causes for the increase in severity of the outbreaks. Of these possibilities, the 3 most likely are an increase in the reservoir of disease because of increased farming activity and/or intensity, increased prevalence and/or severity of a predisposing factor(s) and increased virulence on the part of the virus (e.g. a new strain).

With respect to increased farming activity, the area of shrimp cultivation in Thailand over the interval 1983 to 1991 has risen from approximately 35 000 ha (Menasveta 1990) to 80 000 ha (Rosenberry 1991), while the export of harvested shrimp has risen from approximately 12 000 t (Menasveta 1990) to 110 000 (Rosenberry 1991). With respect to intensity, our experience with stocking densities in Southern Thailand has shown a steady increase over the past few years, from around 30 larvae m^{-2} in 1985 to upwards of 60 or even 100 m^{-2} at the present time. This trend has come about as farmers attempt to compensate for mortality losses during early rearing by increasing the level of initial stocking, even though this practice is discouraged by disease experts.

Turning to predisposing factors, the prime candidate would be stress resulting from environmental deterioration. This includes deterioration in water quality from increased aquaculture activity itself and from increased industrial activity in general. Both types of deterioration have been recently reported in Thailand (Menasveta 1987, 1990, Phillips et al. 1991). The possibility of pesticide residues being implicated has already been mentioned in the 'Introduction'. Indeed, the import of insecticides in Thailand has been increasing steadily over the years (e.g. from 600 to 1400 t of methyl-parathion and from 5 to 35 t of cypermethrin

between 1983 and 1989) (Thai Customs Department unpubl.). Some of these compounds (e.g. the synthetic pyrethroid cypermethrin) are relatively persistent and extremely toxic to *Penaeus monodon*, even at levels of a few $ng\ l^{-1}$ (i.e. parts per trillion) (Flegel et al. 1992).

We may conclude that YBV is one of the most virulent disease agents so far reported for *Penaeus monodon* and that it poses a dire threat to the aquaculture industry for this species. It may also be a threat to other species of commercially cultivated shrimp. Thus, there is an urgent need to carry out further investigations concerning its basic characterization, infectivity, epizootiology and viability under various conditions. Only then will we be able to develop a rational program to control the disease and limit its spread.

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